



INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

I. A. R. I. 6.

MGIPC—SI—6 AR/54—7-7-54—10,000.

東北帝國大學
理科報告
(生物學)
第十六卷

THE
SCIENCE REPORTS
OF THE
TÔHOKU IMPERIAL UNIVERSITY
FOURTH SERIES
(Biology)
SENDAI, JAPAN

Vol. XVI.

On Sale by
MARUZEN COMPANY, LTD., TOKYO AND SENDAI

1941

CONTENTS

No. 1

(Published February 10th, 1941)

	Page
SENJI TANITA	
Report of the Biological Survey of Mutsu Bay. 35. Studies on the Calcareous of Mutsu Bay.	1
MASATO TAHARA	
Further Studies of the Conceptacle Development of <i>Sargassum</i> ...	9
IKUSÔ HAMAI	
A Study of the Growth of the Fish, <i>Cyprinus carpio</i> L.	17
MASATO TAHARA	
Embryogeny of <i>Podocarpus macrophyllus</i> and <i>Podocarpus Nagi</i>	91

No. 2

(Published April 30th, 1941)

KATSUHIRO OKADA	
The Gametogenesis, the Breeding Habits, and the Early Development of <i>Arenicola cristata</i> STIMPSON, a Tubicolous Polychaete. ...	99
SHINJIRO KOBAYASHI	
Earthworms of Korea. II.	147
MITUO SATÔ	
A Comparative Observation of the Hind-Brain of Fish Possessing Barbels, with Special Reference to their Feeding Habits.	157
NOBUO SASAKI	
The Fresh-water Sponges of Tisima-Rettô (the Kurile Islands)....	165
YOSINORI SUGIHARA	
The Embryogeny of <i>Cunninghamia lanceolata</i> HOOKER.	187
MAKOTO TORIUMI	
Studies on Freshwater Bryozoa of Japan. I.	193

ETURÔ HIRAI

- The Early Development of *Cynthia roretzi* DRASCHE. 217

No. 3

(Published August 20th, 1941)

SANJI HÔZAWA AND MAKOTO TORIUMI

- Some Freshwater Bryozoa Found in Manchoukuo. 233

SHINRYÔ OHFUCHI

- The Cavernicolous Oligochaeta of Japan. 243

ETURÔ HIRAI

- * An Outline of the Development of *Cynthia roretzi* DRASCHE. 257

SENJI TANITA

- Calcareous Sponges Obtained from Onagawa Bay and its Vicinity. 263

ISAO MOTOMURA

- Studies of Cleavage II. Cleavage of Eggs of a Sea Urchin, *Strongylocentrotus pulcherrimus*, in Calcium-free Sea Water. 283

YOSINORI SUGIHARA

- Embryological Observations on *Taiwania cryptomerioides* HAYATA. 291

MASA-O YA-E

- Development of the Embryo-Sac in *Statice japonica* SIEBOLD et ZUCCARINI. 297

TOMIO KAKUKAWA

- Über das Redoxpotential des Presssaftes einer Etiolierten Pflanze, *Vicia Faba*, und die Potentialveränderungen einiger Substrate unter der Einwirkung des von der Betreffenden Pflanze Extrahierten Enzyms. 805

HIKOSHICHIRO MATSUMOTO

- Report of the Biological Survey of Mutsu Bay. 36. Ophiuroidea of the Mutsu Bay and Vicinities, 331

ISAO MOTOMURA

- Materials of the Fertilization Membrane in the Eggs of Echinoderms. 345

No. 4

(Published September 30th, 1941)

Page

NOBUO SASAKI

- On the Changes Occurring in Various Parts of the Body, Especially in the Spicules in Accordance with the Increase of Body Length in the Case of the Calcareous Sponge, *Sycon okadai* HÔZAWA. 365

TOHRU UCHIDA

- Actiniaria Collected in the Vicinity of Onagawa Bay. 383

SHINJIRÔ KOBAYASHI

- On Some Earthworms from the South Sea Islands. II. 391

MASATO TAHARA

- Conceptacle Development of Two Species of *Sargassum* of the Subgenus *Micrancantha*. 407

MAKOTO TORIUMI

- Studies on Freshwater Bryozoa of Japan II. Freshwater Bryozoa of Tyôsen (Korea). 413

MASATO TAHARA

- On Some Later Stages of the Embryogeny of *Coccophora Langsdorffi*. 427

ISAO MOTOMURA

- The Sexual Character of the Sea Urchin, *Strongylocentrotus pulcherrimus* A. AGASSIZ. 431

ISAO MOTOMURA

- Artificial Insemination of the Eggs of the Star Fish, *Asterina pectinifera* (MULLER et TROSCHERL). 438

MASATO TAHARA

- On the Structure of the Conceptacle of *Sargassum* and *Coccophora*. 435

KÔGORÔ ABE

- Weitere Untersuchungen über die Befruchtung von *Coccophora* und *Sargassum*. 441

REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY

35. STUDIES ON THE CALCAREA OF MUTSU BAY¹⁾

By

SENJI TANITA

Biological Institute, Tôhoku Imperial University, Sendai, Japan

(With Plate 1 and 2 text-figures)

Received September 27, 1940)

The calcareous sponges of Mutsu Bay have been already reported by Prof. HOZAWA in 1928, dealing with the specimens obtained by the biological survey of this bay. At that time he reported two species of *Leucosolenia mutsu* and *Leucosolenia laxa*. Since then, several specimens have been collected by himself, by Dr. SATO, and by Mr. YAMAMOTO from the same bay. Through the courtesy of these collectors, I was able to have the opportunity of studying the fauna of calcareous sponges of the bay. The collection was found to contain five species, of which one appears to be new to science. In the present paper I shall deal with these species.

Here I should like to express my hearty thanks to Prof. HOZAWA, Dr. SATO, and Mr. YAMAMOTO for their kindness expressed in allowing me to examine their collections.

The following is the list of the species:

Family Homocoelidae

- 1) *Leucosolenia laxa* KIRK
- 2) *Leucosolenia tenera* TANITA

Family Sycettidae

- 3) *Sycon coronatum* (ELLIS and SOLANDER)

Family Grantiidae

- 4) *Leucandra tomentosa* TANITA

Family Amphoriscidae

- 5) *Leucilla minuta*, n. sp.

As is seen from the above list, the number of species treated in the present report is five, and thus the calcarea fauna of Mutsu Bay is represented by six species when the species *Leucosolenia mutsu* HOZAWA is added.

¹⁾ Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 171.

DESCRIPTION OF THE SPECIES

1. *Leucosolenia laxa* KIRK

(Pl. I, fig. 1)

Leucosolenia laxa, KIRK, 1895, p. 208, Pl. IV, fig. 1; DENDY and ROW, 1913, p. 722.
HOZAWA, 1928, p. 220, Pl. I, figs. 4, 5; 1940, p. 35.

This species is represented in the collection by three specimens obtained by Prof. HOZAWA in July, 1929, off Fujishima (St. 116). All of these were found attached by means of their narrowed base to some shells of Brachiopods.

The largest specimen (Pl. I, fig. 1) is ovoid in shape and is more or less laterally compressed. It consists of a massive assemblage of reticulating Ascon-tubes. The total length is 26 mm. and the breadth is 25 mm. The colour is white in alcohol.

The other two specimens are smaller and of a more elongated oval shape than the first, attaining the length of 17 mm. and 8 mm. respectively.

In anatomical structure these specimens agree well with the description given by KIRK of the same species.

Localities: — New Zealand (KIRK); Tairadate and Takaisozaki in Mutsu Bay, Wajima, Ohshima of Rikuzen (HOZAWA); off Fujishima in Mutsu Bay.

2. *Leucosolenia tenera* TANITA

(Pl. I, fig. 2)

Leucosolenia tenera, TANITA, 1940, pp. 166-168, Pl. VIII, fig. 2, textfig. 1.

A single specimen of this species exists in the collection which was taken by Dr. SATO in July, 1929, on the shore of Tsuchiya.

The sponge forms a loose mass of branching Ascon-tubes, attached to the sea weed. The Ascon-tubes are small and thin-walled, some of them bearing a small circular osculum of about 1 mm. diameter at their extremity. The outer surface of the tubes is very minutely hispid. The colour in alcohol is nearly white.

With respect to the inner structure, the present specimen is nearly identical with that of the type.

Localities: — Matsushima Bay (TANITA); Tsuchiya in Mutsu Bay.

3. *Sycon coronatum* (ELLIS and SOLANDER)

Spongia coronata, ELLIS and SOLANDER, 1786, p. 190, Tab. 58, figs. 8, 9.

Sycandra coronata, HAECKEL, 1872, p. 304, Taf. 51, fig. 2, Taf. 60, figs. 1-6.

Sycon coronatum, DENDY, 1892, p. 79; DENDY and ROW, 1913, p. 745; LAUBENFELS, 1932, p. 11; BREITFUSS, 1935, pp. 16-17; HOZAWA, 1940, pp. 140-143, Pl. VI, fig. 5, textfig. 4.

The collection contains one specimen of this species which was taken by Mr. YAMAMOTO by means of a dredge from a depth of about 25 meters off Kawauchi.

The sponge forms a solitary tubular individual. It is broadest near the base and is provided with an osculum surrounded by a well-developed collar at the upper end. It is 13 mm. in total length and 5 mm. in the greatest breadth. The oscular collar is about 6 mm. high and is 1.2 mm. across. The body wall is 1 mm. thick in the middle parts of the body.

The dermal surface is strongly hispid owing to the projecting oxea, while that of the gastral appears nearly smooth to the naked eye. The colour in alcohol is greyish white.

Localities: -- East coast of Australia (HAECKEL, LENDENFELD, DENDY); Atlantic Ocean (HAECKEL, BREITFUSS); Pacific Ocean (HAECKEL); Indian Ocean (ROW); Messina (HOZAWA); off Kawauchi in Mutsu Bay.

4. *Leucandra tomentosa* TANITA

Pl. I, fig. 4)

Leucandra tomentosa, TANITA, 1940, pp. 174-176, Pl. VIII, fig. 6, textfig. 4.

This species is represented by a single specimen. It was secured by Mr. YAMAMOTO in April, 1940, from a depth of about 25 meters off Kawauchi.

The sponge represents a solitary person of an elongated tubular form, showing at the upper end an osculum surrounded by a well-developed collar. The outer surface is strongly hispid on account of the presence of long oxea projecting from it. The gastral cavity is irregular in shape and is rather narrow, the surface of the cavity being smooth. The sponge is 11 mm. high and 4.5 mm. broad in the middle parts. The oscular collar is 2.2 mm high and 1.2 mm. across. The colour in alcohol is greyish white.

In the anatomical structures, the present specimen represents the same features as those of the type.

Localities: -- Matsushima Bay (TANITA); off Kawauchi in Mutsu Bay.

5. *Leucilla minuta*, n. sp.

(Pl. I, fig 5; textfigs. 1, 2)

A single specimen of this new species exists in the collection which was obtained by Prof. HOZAWA in July, 1929, at Igamasaki (St. 111). It (Pl. I, fig. 5) is a small solitary person of an oval shape and somewhat dorsoventrally compressed. It is about 3 mm. in length and 4 mm. in the greatest breadth.

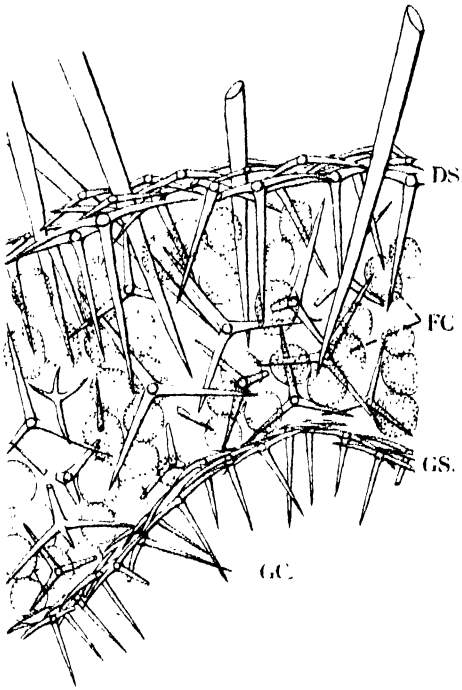
The outer surface is strongly hispid due to the presence of large oxea projecting from it. The osculum at the upper end is nearly circular with a diameter of 1.2 mm. and has a feebly developed oscular fringe. The gastral cavity is relatively narrow and the body wall is 1.2 mm. thick in the middle parts. The gastral surface is also hispid from the projecting apical rays of the gastral quadriradiates.

The colour in alcohol is white.

Structure (Textfig. 1):—

The canal system is of the leuconoid. The flagellate chambers (FC) are either spherical or ovoid and are thickly packed in the chamber layer.

The skeleton of the dermal cortex (DS) is composed of triradiates, the facial rays of subdermal quadriradiates, and large oxea. The triradiates lie tangentially in a thin layer and the facial rays of the subdermal quadriradiates are also placed tangentially in a rather confused arrangement. The large oxea which occur fairly thickly in the sponge wall project from the dermal surface making nearly right angles with it, but nearer the osculum these spicules have a



Textfig. 1. *Leucilla minuta*, n. sp. Part of a cross-section ($\times 65$). DS, Dermal skeleton; FC, flagellate chambers; GC, gastral cavity; GS, gastral skeleton.

tendency to be placed parallel to the long axis of the sponge.

The tubar skeleton is made up of the apical rays of subdermal quadriradiates and of tubar quadriradiates arranged in several confused layers with their basal rays pointing centrifugally. The proximal parts of large oxea and the basal rays of subgastral quadriradiates may be added to the skeleton.

The gastral skeleton (GS) is thinner than that of the dermal and consists mainly of tangentially placed gastral quadriradiates.

The skeleton of the oscular margin is a close interlacement of linear spicules and quadriradiates with strongly divergent paired rays and downwardly directed basal rays. The former kind of spicules are arranged longitudinally. There may be also found in addition some large oxea disposed parallel to the long axis of the sponge.

Spicule (Textfig. 2) : Dermal triradiates (a) slightly sagittal. Basal ray straight, either equal to or slightly shorter than the paired rays, $150-240\ \mu$ long and $12-16\ \mu$ thick at base. Paired rays nearly equal, slightly curved forwards, $170-240\ \mu$ long and $12-16\ \mu$ thick at base.

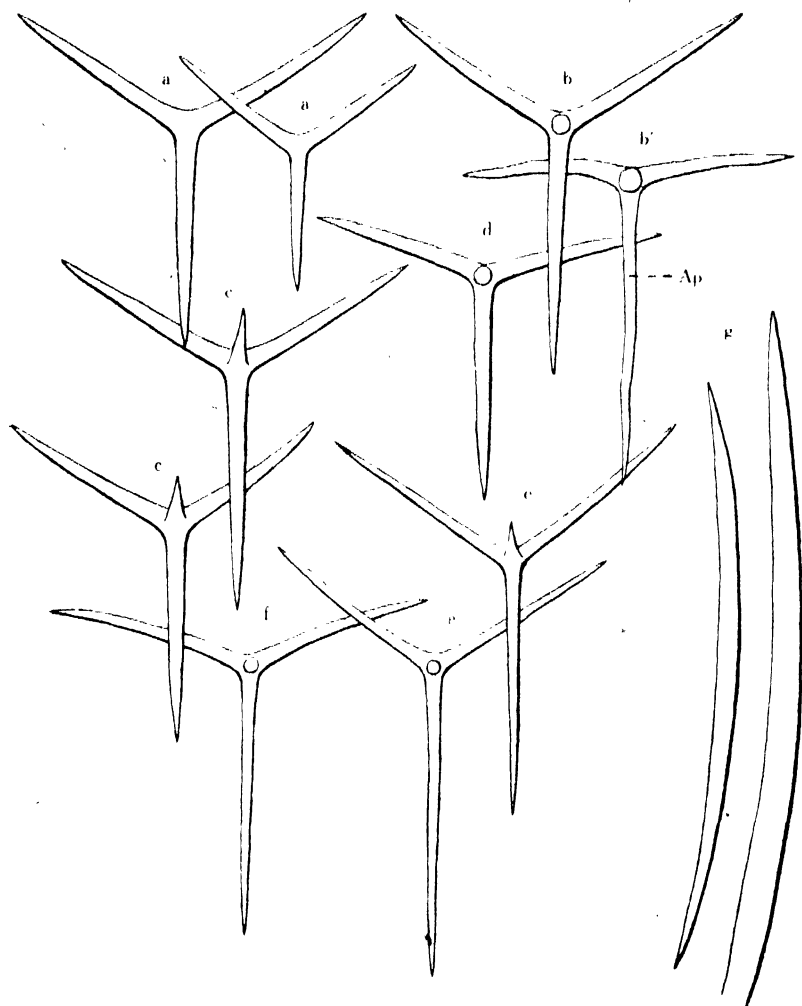
Subdermal quadriradiates (b) sagittal, all rays of nearly equal thickness. Basal ray straight, sharply pointed, slightly longer than paired rays, $140-220\ \mu$ long and $12-16\ \mu$ thick at base. Paired rays equal, slightly curved forwards, $110-210\ \mu$ long and $12-16\ \mu$ thick at base. Apical ray straight or slightly curved, sharply pointed, longer than facial rays, $180-330\ \mu$ long and $12-16\ \mu$ thick at base.

Tubar quadriradiates (c) also slightly sagittal, rays stout. Basal ray straight, gradually tapering, sharply pointed, $240-360\ \mu$ long and $15-20\ \mu$ thick at base. Paired rays nearly equal, slightly shorter than basal ray, $200-330\ \mu$ long and $15-20\ \mu$ thick at base. Apical ray much shorter than facial rays, straight or slightly curved, sharply pointed, about $90\ \mu$ long and $12-18\ \mu$ thick at base.

Subgastral quadriradiates (d) exactly similar to that of the tubar, but having the greater oral angles.

Gastral quadriradiates (e) sagittal. Basal ray straight, longer than paired rays, gradually tapering to sharp end, $260-340\ \mu$ long and $10-14\ \mu$ thick at base. Paired rays either equal or unequal, slightly curved forwards, $240-300\ \mu$ long and $10-14\ \mu$ thick at base. Apical ray shorter and slightly thinner than facial rays, straight or slightly curved upwards, $100-210\ \mu$ long and $8-12\ \mu$ thick at base.

Quadriradiates of the oscular margin (f) similar to the quadriradiates of the gastral cortex, differing only in having the wider oral angles.



Textfig. 2. *Leucilla minuta*, n. sp. a, dermal triradiates; b, subdermal quadriradiate; b', the same seen from lateral side; c, tubar quadriradiates; d, subgastral quadriradiate; e, gastral quadriradiates; f, quadriradiate of oscular margin; g, oxea of dermal surface; Ap, Apical ray. (all $\times 150$)

Large oxea projecting from dermal surface (g) elongate spindle-shaped, slightly curved, sharply pointed at both ends, 570μ – 1.5 mm. long and 25 – 37μ thick in the thickest parts.

Linear spicules of the oscular margin straight, nearly uniformly thick throughout their greater length, measuring 2 – 6μ in thickness. They are

solely sharply pointed at the proximal end while most of the free ends are broken off.

Remarks: - In external form this species bears a marked resemblance to *Leucilla lanceolata* ROW and HOZAWA¹, while in spiculation it approaches to *L. princeps* ROW and HOZAWA². *Leucilla lanceolata* differs from the present species in the absence of dermal triradiates and also in the presence both of tubar triradiates and lance headed oxea. From *L. princeps* the present species may be easily distinguished by the external features and by the differences seen in the shape of subdermal quadriradiates and of tubar quadriradiates. Moreover the present species differs from these two species in the absence of trichoxea.

Locality: - Igamasaki in Mutsu Bay.

LITERATURE CITED

- BREITFUSS, L. (1935) Le Spungne calcaree dell' Adriatico con riflesso a tutto il Mediterraneo. Consiglio Nazionale Ricerche R. Comitato Talassografico-Italiano, Memoria CCXXIII.
- DENDY, A. (1892) Synopsis of the Australian Calcareous Heterocoela, with a Proposed Classification of the Group, and Description of Some New Genera and Species. Proc. Roy. Soc. Victoria (n.s.) Vol. V, pp. 69-116.
- DENDY, A. and ROW, W. H. (1913) The Classification and Phylogeny of the Calcareous Sponges, with a Reference List of all the described Species, Systematically Arranged. Proc. Zool. Soc. London, 1913, pp. 704-813.
- ELLIS, J. and SOLANDER, D. (1786) Natural History of Many Curious and Uncommon Zoophytes collected from Various Parts of the Globe. London 1786.
- HAECKEL, E. (1872) Die Kalkschwämme, eine Monographie, Berlin.
- HOZAWA, S. (1928) Report of the Biological Survey of Mutsu Bay. 6. Calcareous of Mutsu Bay. Sci. Rep. Tōhoku Imp. Univ. Ser. Biol. Vol. III, No. 2, pp. 219-222, Pl. I.
- (1940) On Some Calcareous Sponges from Japan. Sci. Rep. Tōhoku Imp. Univ. Ser. Biol. Vol. XV, No. 1, pp. 29-58, Pls. IV, V, Textfigs. 9.
- (1940) Report on the Calcareous Sponges obtained by the Zoological Institute and Museum of Hamburg. Sci. Rep. Tōhoku Imp. Univ. Ser. Biol. Vol. XV, No. 2, pp. 131-163, Pls. VI, VII, Textfigs. 10.
- KIRK, H. B. (1895) New Zealand Sponges. Third Paper. Trans. New Zealand Inst., Vol. XXVIII, pp. 205-210, Pls. III, IV.
- LAUBENFELS, M. W. (1932) The Marine and Fresh-water Sponges of California. Proc. U. S. Nat. Mus., Vol. 81, pp. 1-140.
- ROW, R. W. and HOZAWA, S. (1931) Report on the Calcareous obtained by the Hamburg South West Australian Expedition of 1905. Sci. Rep. Tōhoku Imp. Univ. Ser. Biol., Vol. VI, No. 4, pp. 727-809, Pls. XIX-XXI, Textfigs. 17.
- TANITA, S. (1940) Calcareous Sponges of Matsushima Bay. Sci. Rep. Tōhoku Imp. Univ. Ser. Biol., Vol. XV, No. 2, pp. 165-177, Pl. VIII, Textfigs. 4.

¹*Leucilla lanceolata* ROW and HOZAWA, 1931, pp. 795-798, Pl. XXI, fig. 16, textfig. 15.

²*Leucilla princeps* ROW and HOZAWA, 1931, pp. 799-802, Pl. XXI, fig. 17, textfig. 16.

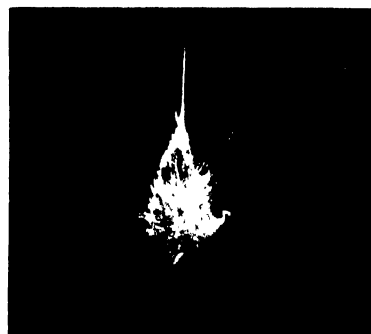
EXPLANATION OF PLATE I.

- Fig. 1. *Leucosolenia laxa* KIRK. Natural size.
Fig. 2. *Leucosolenia tenera* TANITA, $\times 2$
Fig. 3. *Sycon coronatum* (ELLIS and SOLANDER), $\times 2$.
Fig. 4. *Leucandra tomentosa* TANITA, $\times 2$.
Fig. 5. *Leucilla minuta*, n. sp. $\times 2$.

3



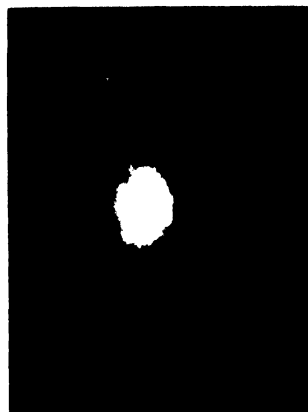
1



12



5



TANITA photo.

S. TANITA: Calcareous of Mutsu Bay.

FURTHER STUDIES OF THE CONCEPTACLE DEVELOPMENT OF SARGASSUM

By

MASATO TAHARA

Biological Institute, Tôhoku Imperial University, Sendai, Japan

(With 10 Text-figures)

(Received October 8, 1940)

In a previous paper (1940) the present writer described the conceptacle development of two species of *Sargassum*, namely *S. Horneri* and *S. enerve*. In these two species the initial cell of the conceptacle is divided at first by a curved wall into two cells, the upper one being the tongue cell. The lower cell repeats the longitudinal divisions and forms the inner wall of the conceptacle. Later the tongue cell becomes free from the wall of the conceptacle and is transferred to the opening of the conceptacle. Thus the opening of the conceptacle is for a time almost completely closed from the outside. In 1906 Miss SIMONS published a paper on the development of the conceptacle of *Sargassum filipendula*. But she makes no allusion to the translocation of the tongue cell in this species. So it has now become desirable to ascertain, how common is the translocation of the tongue cell in all the species of *Sargassum*. Fortunately a large number of *Sargassum* species grow along the coast of Japan; in the present studies eleven of them were investigated in this respect. The writer wishes to express his hearty thanks to Mr. S. INOUE and Mr. K. ABE for securing the material.

1. *Sargassum hemiphyllum* Ag.

The material of this plant was obtained on April 21st of this year 1940 in Misaki. The receptacle is rather small. But a cross section through the terminal portion of a thallus, where a number of young conceptacles are found clustered together, reveals in profusion different stages of the conceptacle development. The procedure of the conceptacle development in this plant agrees with that of *S. enerve* described in the previous paper. The translocation of the tongue cell occurs in an early stage of its development. Secretion of a gelatinous substance around the tongue cell is conspicuous (Fig. 1).

2. *Sargassum tortile* AG.

The material of this plant was obtained also on April 21st of this year in Misaki. The conceptacle development is similar to that in the preceding species. The translocation of the tongue cell is shown in Fig. 2.

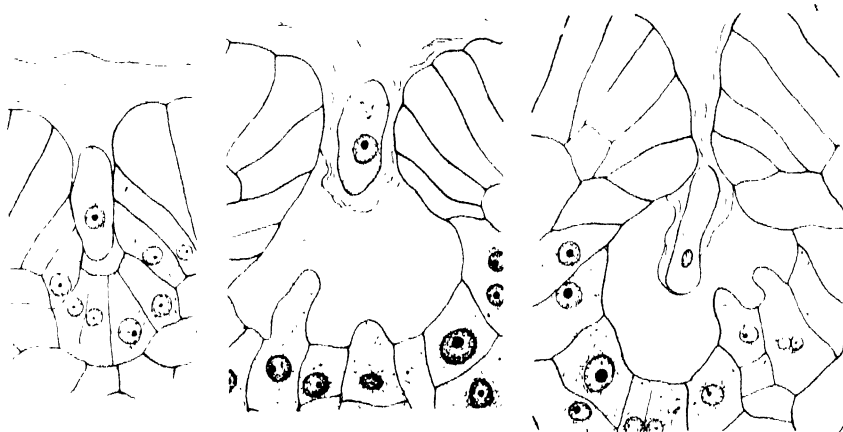


Fig. 1. a.

Fig. 1. b.

Fig. 2.

Fig. 1. a, b. *Sargassum hemiphyllum*. $\times 850$. Fig. 2. *Sargassum tortile*. $\times 850$.

3. *Sargassum confusum* AG.

The material of this alga was collected by Mr. K. ABE at Asamushi in early May of this year. The conceptacle development agrees in substance with that of the former species (Fig. 3).

4. *Sargassum Ringgoldianum* HARV.

This species has a number of unique characteristics. But the conceptacle development is nearly similar to that already described (Fig. 4). One point, however, is worth mentioning: the tongue cell of this plant shows signs of degeneration in an early stage of its development. The material of this plant was collected by the writer on June 7th of this year in Misaki.

5. *Sargassum sagamianum* YENDO

This species has also a series of unique characteristics. Receptacles

attain maturity in August. The material of the present investigation was collected by the writer on August 6th of this year in Enoshima. The dislocation of the tongue cell from the wall of the conceptacle occurs in early stage of the development (Fig. 5).

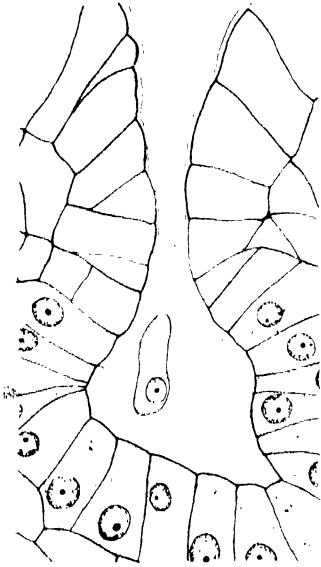


Fig. 3.

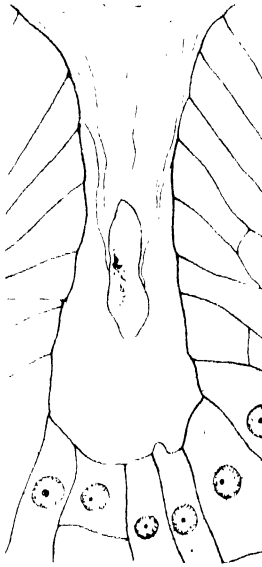


Fig. 4.

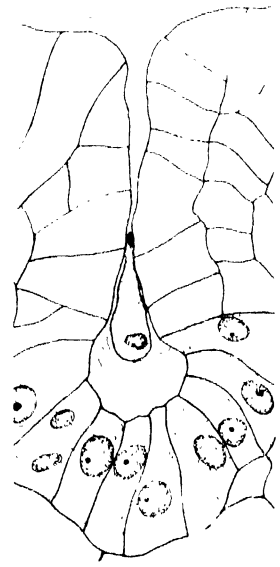


Fig. 5.

Fig. 3. *Sargassum confusum*. $\times 850$. Fig. 4. *Sargassum Ringgoldianum*. $\times 850$.

Fig. 5. *Sargassum sagamianum*. $\times 850$.

6. *Sargassum Thunbergii* O. KUNTZE

This plant is one of the most common species of *Sargassum* found in Japan. The material of this plant was collected by Mr. K. ABE at Asamushi early July of this year. The conceptacle development shows no difference to those already described (Fig. 6).

7. *Sargassum fusiforme* (HARV.) SETCH.

Sargassum fusiforme (HARV.) SETCH. is also an alga very common along the coast of Japan. This plant was described at first by HARVEY (1859) as a species of *Cystophyllum*. Later YENDO (1907), with some hesitation, transferred the plant under the genus *Turbinaria*. More recently, however, OKAMURA (1932) segregated this alga as a species of

a new genus *Hizikia*. But one year later SETCHELL (1933) placed this species under the genus *Sargassum*. Under such circumstances it seems necessary to study the conceptacle development of this plant.

This plant attains maturity in June. The material of this alga was sent by Mr. INOH who in May this year visited Amakusa Marine Biological Station of the Kiushu Imperial University. He collected the material on May 10th on the sea shore at Tomioka in the Amakusa Islands.

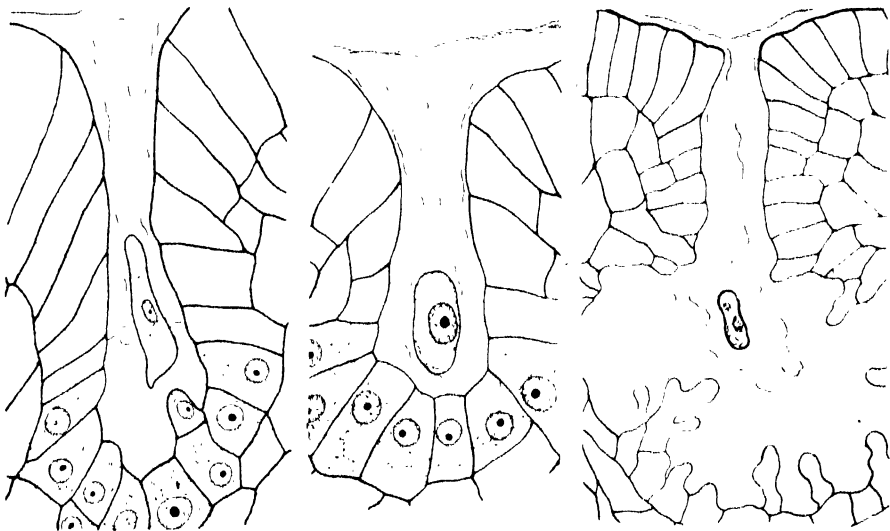


Fig. 6.

Fig. 7. a.

Fig. 7. b.

Fig. 6. *Sargassum Thunbergii*. $\times 850$.

Fig. 7, a, b. *Sargassum fusiforme*. a, $\times 850$. b, $\times 470$.

As Fig. 7 shows, the conceptacle development of this alga is practically similar to that of the species of *Sargassum* already described. It seems correct to the writer to place this plant under the genus *Sargassum*.

8. *Sargassum piluliferum* Ag.

As is well known, the genus *Sargassum* is divided into six subgenera, namely *Phyllotricha*, *Schizophycus*, *Eusagassum*, *Bactrophycus*, *Micracantha* and *Arthrophycus*. The two species described in the previous paper and seven species hitherto treated in the present paper all belong to the subgenus *Bactrophycus*. *Sargassum piluliferum*, the conceptacle development of which will be described now, belongs to the subgenus *Phyllotricha*. The material of this plant was collected by the writer on April 21st of this year in Misaki.

It is remarkable that the conceptacle development of this species is totally different from that of those species belonging to the subgenus *Bactrophycus*. The tongue cell of this plant is formed, as usual, by the division of the initial cell, but after its formation it soon becomes degenerated

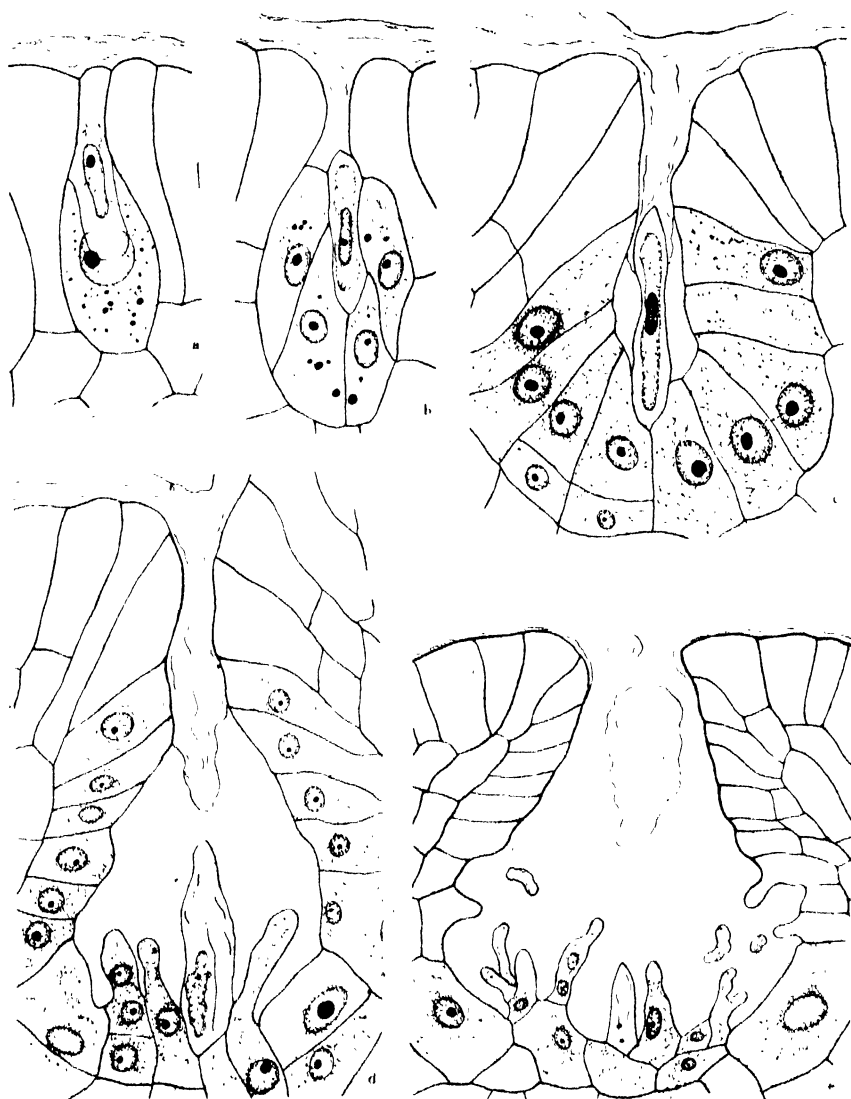


Fig. 8. *Sargassum piluliferum*. a, the initial cell is divided into two cells. b-c, the lower cell is repeatedly divided by longitudinal walls. d-e, further stages of development. a-d, $\times 850$. e, $\times 470$.

and remains attached to the wall of the conceptacle (Fig. 8). The translocation of the tongue cell commonly seen in the species of *Bactrophycus* never takes place in the present species.

9. *Sargassum patens* Ag.

This species belongs to the subgenus *Schizophycus*. The material of this plant was collected by the writer on June 7th of this year in Misaki. The conceptacle development has much resemblance to that of *S. piluliferum*. The translocation of the tongue cell does not occur also in this plant (Fig. 9).

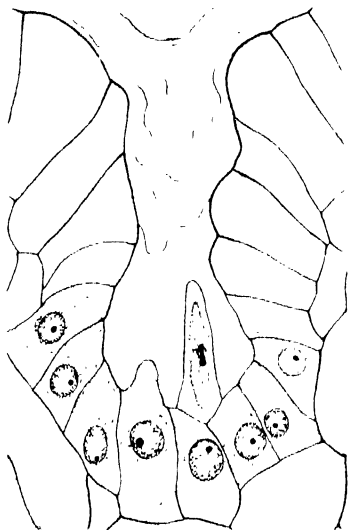


Fig. 9. *Sargassum patens*. $\times 850$.

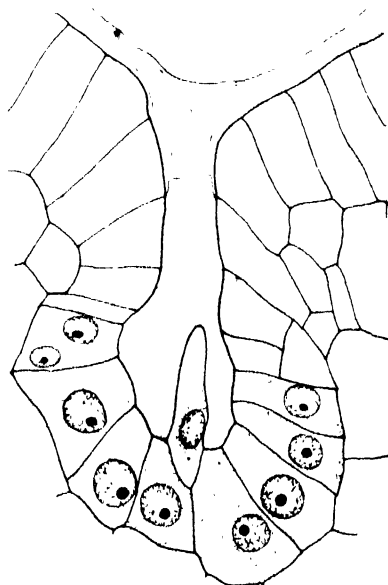


Fig. 10. *Sargassum duplicatum*. $\times 850$.

10. *Sargassum duplicatum* J. Ag.

This species belongs to the subgenus *Eusargassum*. *Sargassum filipendula*; which was the material of Miss Simons' investigation, belongs also to the subgenus *Eusargassum*. So the study of a species of this subgenus is very important. Fortunately the writer was able to collect the material of *S. duplicatum* on July 6th in Enoshima, Kanagawa Prefecture. The conceptacle development takes place in the same manner as described by Miss SIMONS. The tongue cell becomes degenerated and remains fixed to the wall of the conceptacle (Fig. 10).

The material of a species belonging also to the subgenus *Eusargassum* was sent by Mr. INOH, who collected it on May 21st of this year on the sea shore near Ushibuka in the Amakusa Islands. The specific name of this plant is unfortunately unknown to the writer at present. The conceptacle development is carried out as in the species above described.

SUMMARY AND CONCLUSION

1. The conceptacle development of eleven species of *Sargassum* was followed in the present investigation. Among these species seven belong to the subgenus *Bactrophycus*, one to the subgenus *Phyllotricha*, also one to the subgenus *Schizophycus* and two to the subgenus *Eusargassum*.

2. The translocation of the tongue cell towards the opening of the conceptacle was observed in the seven species of *Bactrophycus*.

3. In the species of the subgenera, *Phyllotricha*, *Schizophycus* and *Eusargassum* the translocation of the tongue cell does not occur. The tongue cell becomes degenerated in an early stage of its development and remains fixed to the wall of the conceptacle.

4. The translocation of the tongue cell appears to be one of the most important characteristics of the more advanced groups of *Sargassum*.

5. Divided leaves of *Phyllotricha* and *Schizophycus* and the ramification of the receptacles of *Eusargassum* are the signs of the primitiveness of these subgenera, in which translocation of tongue cell cannot be observed.

LITERATURE

- OKAMURA, K. (1932). *Icones of Japanese Algae*. Vol. 6. No. 10.
SIMONS, E. B. (1906). A Morphological Study of *Sargassum filipendula*. Bot. Gaz. 41.
SETCHELL, W. A. (1932). Hongkong Seaweeds, III Sargassaceae. Hongkong Naturalist Supplement.
TAHARA, M. (1940). On the Development of the Conceptacle of *Sargassum*, *Coccophora* and *Cystophyllum*. Sci. Rep. Tôhoku Imp. Univ. Vol. 15. No. 3.
YENDO, K. (1907). The Fucaceae of Japan. Jour. Coll. Sci. Imp. Univ. Tokyo. Vol. 21.

A STUDY OF THE GROWTH OF THE FISH, *CYPRINUS CARPIO* L.

By

IKUSÔ HAMAI

The East Asia Institute, Surugudai, Kanda, Tôkyô

(With 13 Figures)

Received October 17, 1940

CONTENTS

I. Introduction.....	18
II. Material and Method	18
1. Material Specimen.....	18
2. Diet	19
3. Measurement	19
III. Absolute Growth	28
1. General.....	28
2. Effect of Age on Growth	31
3. Effect of Size on Growth	36
4. Effect of Diet on Growth	37
IV. Individual Growth	37
1. General.....	37
2. Absolute Growth	38
3. Relative Growth.....	45
V. Relative Growth	53
1. Relative Growth-Rate and its Ratio.....	53
2. Effect of Age on Relative Growth	59
3. Effect of Size on Relative Growth	60
4. Growth Gradient	61
5. Effect of Diet on Relative Growth	64
VI. Variation of Size	65
1. General.....	65
2. Age, Size, and Direction of Variation	68
3. Growth and Variation in Size	70
VII. Discussions	73
1. Natural Growth	73
2. Relative Growth.....	76
3. Thickness of the Body	80
4. Physiologic Age	82
5. Variation in Size and the Direction-Coefficient of Variation.....	83
VIII. Summary	84
IX. References	87

I. INTRODUCTION.

Up to the present time, the study of the relative growth of fish has been carried out by many workers. But, from the point of view of the relative growth-rate, with special reference to the growth-gradient, few data have been reported so far as I know. In the problems of relative growth, the important factors, *e. g.* the time and the size, have been the main objects for study with the problems of physiological foundation. In many investigations regarding relative growth, the time-factor has been neglected. The determination of growth by means of the size only is less accurate on account of the great variation in size, an inevitable characteristic of living beings. Here a question arises about the relation between the growth and the variation in size. The features of growth affected by the age-factor and by the size-factor, and the relationship between such growth and variation in size are necessarily important and interesting subjects requiring a clear explanation.

HAMAI (1937) has partially succeeded in adding the time-factor in the analysis of relative growth in a certain Mollusca. BRODY (1937) has suggested the relativity of time and mass. To make clear a part of these problems, especially regarding the growth of fish, is the purpose of this paper. This subject regarding the growth of fish was suggested by Prof. E. NOMURA several years ago.

Here, I wish to express my sincere thanks to Prof. E. NOMURA for his kind guidance during a long course of time, and I am much indebted to Mr. KATSUHIRO OKADA for the advance of this investigation.

II. MATERIAL AND METHOD.

1. Material Specimen.

Cyprinus carpio is a fresh-water fish reared for commercial purposes widely in Japan, and which is naturally, also, distributed widely in Japan. The material specimens were brought from the fish ponds near Sendai, in which they were bred. Experiments were begun on June 30th, 1936 and finished on January 10th, 1939.

The material specimens consisted of several groups, the larval fishes hatched out on June 27th, 1936, one year old fishes, and two year old fishes. On August 13th, 1936, new materials were added, which consisted of the fishes born in that year and those born in 1935. The groups were separately reared in different ponds. The number of fishes, size of ponds, designation of groups, etc. are tabulated in Table 1. From this

point, the groups of fish will, for convenience, be called by the representative designations in the Table.

TABLE 1.

Group	Year, born in	Number of Specimens		Size of pond length \times breadth \times depth (cm)	Remarks
		Beginning of Exp.	End of Exp.		
I	1936, June 27	30	—	—	
II	1936	19	6	147 \times 90 \times 20	
III	1935	20	11	135 \times 70 \times 20	Smaller specimens.
IV	1935	10	7	182 \times 93 \times 50	Larger specimens.
V	1934	25	—	—	Each 5 taken in two ponds of the same size.
VI	1938	17	9	145 \times 90 \times 20	

The specimens born in 1935 consisted of two groups, one of the larger, and the other of those which had remained small up to the beginning of the experiment. This condition of size continued until the end of the experiments.

2. Diet.

Both artificial and natural diets were given. As the artificial food, the dried pupae of the silk-worm from the cocoon were given, a food which is ordinarily supplied to the carp reared in fish-ponds, and also a food, consisting of fish-meal and flour in equal quantities, to which are added some minced vegetable leaves and sometimes some table-salt, was given. This mixed food is very suitable for rearing the carp and for its growth. Many fishery-breeders give this food successfully. As the natural food, some kinds of earth-worm were given.

The food was given once every other day, the quantity being as much as the fish could devour. After October 7th, 1937, the amount of diet was diminished by half, and the influence of this on the growth was observed. No diet was given after August 20th, 1938 until the end of the experiments.

3. Measurement.

During the course of the investigation measurements of length-growth were taken several times. At the time of the measurements the fishes were narcotized by chloretone. In the adult fish, the following measurements were taken: the straight length from the frontal end of the snout to the back end of the eye (*A*), from the back end of the eye to the back end of the gill-cover (*B*), from the back end of the gill-cover to the frontal end of the dorsal fin (*C*), from the frontal end of the dorsal

fin to the anus (D), and from the anus to the last scale on the lateral line (E), respectively along the axial line of the body (Fig. 1, A).

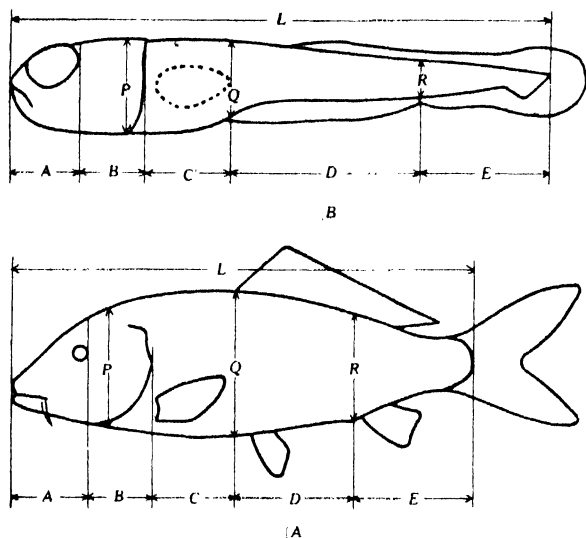


Fig. 1. Method of measurements. Above [B]: larval fish, below [A]: adult fish.

In the larval fish, the length from the back end of the gill-cover to the back end of the air-bladder (C') was measured, in place of the C -measurement of the adult fish, the dorsal fin not being clearly observable. The other measurements were the same as in the adult (Fig. 1, B). The depth of the fish was measured in three places, the head-depth (P), the depth at the frontal end of the dorsal fin (Q), and at the anus (R) in the adult fish. In the larval fish, the middle depth was taken at the back end of the air-bladder (Q') instead of at the frontal end of the dorsal fin (Q) in the adult fish. The other two depths were at the same places as in the adult (Fig. 1).

The width of the body was measured at the same points as those at which the measurements of the depth were taken. From the frontal part, the letters of X , Y and Z , stand for each width respectively. The width measurements were only taken in the adult fish, but not in the larval fish. In both the adult and the larval fish, the standard length (L), from the frontal end of the snout to the last scale on the lateral line, was measured, and this length was made as the standard of measurements and of calculations.

The measurements are shown in Tables 2-14, and 21.

TABLE 2.
Growth of Standard Length (L) (cm.).

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	Standard length (L)	Date of measurements	No. of obs.	Standard length (L)	Date of measurements	No. of obs.	Standard length (L)
0	Oct. 1, 1936	19	5.84 ± 0.14						
1	July 26, 1937 Oct. 6, 1937	5 5	10.85 ± 0.45 14.27 ± 0.55	Oct. 7, 1936	6	8.56 ± 0.24	Sept. 29, 1936	10	19.66 ± 0.24
2	Aug. 18, 1938 Jan. 5, 1939	5 5	15.01 ± 0.61 15.16 ± 0.68	July 27, 1937 Oct. 6, 1937	6 6	11.20 ± 0.56 14.76 ± 0.89	July 26, 1937 Oct. 4, 1937	10 9	23.07 ± 0.28 27.01 ± 0.37
3				Aug. 20, 1938 Jan. 6, 1939	6 6	15.21 ± 0.93 15.20 ± 0.92	Aug. 17, 1938 Jan. 5, 1939	7 7	26.22 ± 0.23 26.17 ± 0.26

TABLE 3.
Growth of the dimension from the frontal end of snout to the back end of eye (A) (cm.).

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	A	Date of measurements	No. of obs.	A	Date of measurements	No. of obs.	A
0	Oct. 1, 1936	19	1.00 ± 0.02						
1	July 26, 1937 Oct. 6, 1937	5 5	1.66 ± 0.05 2.05 ± 0.07	Oct. 7, 1936	6	1.37 ± 0.04	Sept. 29, 1936	10	2.75 ± 0.03
2	Aug. 18, 1938 Jan. 5, 1939	5 5	2.14 ± 0.06 2.25 ± 0.07	July 27, 1937 Oct. 6, 1937	6 6	1.73 ± 0.05 2.02 ± 0.09	July 26, 1937 Oct. 4, 1937	10 9	2.96 ± 0.04 3.29 ± 0.06
3				Aug. 20, 1938 Jan. 6, 1939	6 6	2.16 ± 0.09 2.21 ± 0.08	Aug. 17, 1938 Jan. 5, 1939	7 7	3.15 ± 0.06 3.17 ± 0.04

TABLE 6.
Growth of the dimension measured from the frontal end of dorsal fin to the anus (D) (cm.).

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	D	Date of measurements	No. of obs.	D	Date of measurements	No. of obs.	D
0	Oct. 1, 1936	19	1.56 ± 0.04						
1	July 26, 1937 Oct. 6, 1937	5 5	3.11 ± 0.14 4.17 ± 0.17	Oct. 7, 1936	6	2.26 ± 0.05	Sept. 29, 1936	10	5.54 ± 0.07
2	Aug. 18, 1938 Jan. 5, 1939	5 5	4.12 ± 0.21 4.02 ± 0.20	July 27, 1937 Oct. 6, 1937	6 6	2.78 ± 0.15 3.97 ± 0.30	July 26, 1937 Oct. 4, 1937	10 9	6.40 ± 0.12 7.67 ± 0.11
3				Aug. 20, 1938 Jan. 6, 1939	6 6	3.99 ± 0.24 3.93 ± 0.30	Aug. 17, 1938 Jan. 5, 1939	7 7	7.72 ± 0.14 7.41 ± 0.11

TABLE 7.
Growth of the dimension measured from the anus to the last scale on the lateral line (E) (cm.).

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	E	Date of measurements	No. of obs.	E	Date of measurements	No. of obs.	E
0	Oct. 1, 1936	19	1.40 ± 0.03						
1	July 26, 1937 Oct. 6, 1937	5 5	2.54 ± 0.13 3.68 ± 0.19	Oct. 7, 1936	6	2.26 ± 0.08	Sept. 29, 1936	10	5.33 ± 0.07
2	Aug. 18, 1938 Jan. 5, 1939	5 5	3.90 ± 0.17 4.08 ± 0.20	July 27, 1937 Oct. 6, 1937	6 6	3.09 ± 0.18 4.07 ± 0.26	July 26, 1937 Oct. 4, 1937	10 9	6.55 ± 0.09 7.58 ± 0.10
3				Aug. 20, 1938 Jan. 6, 1939	6 6	4.34 ± 0.30 4.41 ± 0.27	Aug. 17, 1938 Jan. 5, 1939	7 7	7.42 ± 0.08 7.58 ± 0.07

TABLE 8.
Growth of depth measured at the head-part (P) (cm.).

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	P	Date of measurements	No. of obs.	P	Date of measurements	No. of obs.	P
0	Oct. 1, 1936	19	1.53 ± 0.05						
1	July 26, 1937 Oct. 6, 1937	5 5	2.60 ± 0.03 3.25 ± 0.13	Oct. 7, 1936	6	2.05 ± 0.06	Sept. 29, 1936	10	4.50 ± 0.04
2	Aug. 18, 1938 Jan. 5, 1939	5 5	3.44 ± 0.10 3.40 ± 0.14	July 27, 1937 Oct. 6, 1937	6 6	2.48 ± 0.11 3.30 ± 0.17	July 26, 1937 Oct. 4, 1937	10 9	4.86 ± 0.05 5.71 ± 0.10
3				Aug. 20, 1938 Jan. 6, 1939	6 6	3.32 ± 0.17 3.23 ± 0.18	Aug. 17, 1938 Jan. 5, 1939	7 7	5.49 ± 0.07 5.66 ± 0.08

TABLE 9.
Growth of depth measured at the frontal end of dorsal fin (Q) (cm.).

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	Q	Date of measurements	No. of obs.	Q	Date of measurements	No. of obs.	Q
0	Oct. 1, 1936	19	2.05 ± 0.06						
1	July 26, 1937 Oct. 6, 1937	5 5	4.08 ± 0.18 4.96 ± 0.20	Oct. 7, 1936	6	2.90 ± 0.06	Sept. 29, 1936	10	6.16 ± 0.09
2	Aug. 18, 1938 Jan. 5, 1939	5 5	4.84 ± 0.20 4.60 ± 0.19	July 27, 1937 Oct. 6, 1937	6 6	3.62 ± 0.16 4.92 ± 0.25	July 26, 1937 Oct. 4, 1937	10 9	7.45 ± 0.10 8.58 ± 0.16
3				Aug. 20, 1938 Jan. 6, 1939	6 6	4.59 ± 0.23 4.24 ± 0.23	Aug. 17, 1938 Jan. 5, 1939	7 7	7.71 ± 0.11 7.48 ± 0.11

TABLE 10. *Growth of depth measured at the point of anus (R) (cm.).*

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	R	Date of measurements	No. of obs.	R	Date of measurements	No. of obs.	R
0	Oct. 1, 1936	19	1.34 ± 0.04						
1	July 26, 1937 Oct. 6, 1937	5 5	2.65 ± 0.11 3.40 ± 0.14	Oct. 7, 1936	6	2.09 ± 0.05	Sept. 29, 1936	10	4.46 ± 0.06
2	Aug. 18, 1938 Jan. 5, 1939	5 5	3.43 ± 0.15 3.31 ± 0.16	July 27, 1937 Oct. 6, 1937	6 6	2.65 ± 0.14 3.52 ± 0.20	July 26, 1937 Oct. 4, 1937	10 9	5.38 ± 0.06 6.28 ± 0.12
3				Aug. 20, 1938 Jan. 6, 1939	6 6	3.27 ± 0.19 3.17 ± 0.20	Aug. 17, 1938 Jan. 5, 1939	7 7	5.58 ± 0.10 5.68 ± 0.10

TABLE 11. *Growth of width measured at the head-part (X) (cm.).*

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	X	Date of measurements	No. of obs.	X	Date of measurements	No. of obs.	X
0	Oct. 1, 1936	19	1.19 ± 0.03						
1	July 26, 1937 Oct. 6, 1937	5 5	2.02 ± 0.08 2.68 ± 0.12	Oct. 7, 1936	6	1.62 ± 0.04	Sept. 29, 1936	10	3.56 ± 0.07
2	Aug. 18, 1938 Jan. 5, 1939	5 5	2.69 ± 0.10 2.78 ± 0.13	July 27, 1937 Oct. 6, 1937	6 6	1.96 ± 0.08 2.61 ± 0.16	July 26, 1937 Oct. 4, 1937	10 9	3.94 ± 0.05 4.98 ± 0.07
3				Aug. 20, 1938 Jan. 6, 1939	6 6	2.66 ± 0.14 2.65 ± 0.16	Aug. 17, 1938 Jan. 5, 1939	7 7	4.63 ± 0.08 4.68 ± 0.09

TABLE 12.
Growth of width measured at the frontal end of dorsal fin (Y) (cm.).

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	Y	Date of measurements	No. of obs.	Y	Date of measurements	No. of obs.	Y
0	Oct. 1, 1936	19	1.13 ± 0.04						
1	July 26, 1937 Oct. 6, 1937	5 5	2.33 ± 0.09 2.77 ± 0.11	Oct. 7, 1936	6	1.57 ± 0.04	Sept. 29, 1936	10	3.64 ± 0.08
2	Aug. 18, 1938 Jan. 5, 1939	5 5	2.66 ± 0.11 2.48 ± 0.10	July 27, 1937 Oct. 6, 1937	6 6	1.92 ± 0.10 2.80 ± 0.15	July 26, 1937 Oct. 4, 1937	10 9	4.42 ± 0.08 5.19 ± 0.10
3				Aug. 20, 1938 Jan. 6, 1939	6 6	2.53 ± 0.15 2.27 ± 0.15	Aug. 17, 1938 Jan. 5, 1939	7 7	4.62 ± 0.09 4.49 ± 0.09

TABLE 13.
Growth of width measured at the point of anus (Z) (cm.).

Age	Group II.			Group III.			Group IV.		
	Date of measurements	No. of obs.	Z	Date of measurements	No. of obs.	Z	Date of measurements	No. of obs.	Z
0	Oct. 1, 1936	19	0.55 ± 0.02						
1	July 26, 1937 Oct. 6, 1937	5 5	1.04 ± 0.04 1.44 ± 0.06	Oct. 7, 1936	6	0.89 ± 0.03	Sept. 29, 1936	10	1.40 ± 0.02
2	Aug. 18, 1938 Jan. 5, 1939	5 5	1.55 ± 0.07 1.38 ± 0.07	July 27, 1937 Oct. 6, 1937	6 6	1.06 ± 0.05 1.56 ± 0.09	July 26, 1937 Oct. 4, 1937	10 9	2.42 ± 0.03 2.79 ± 0.05
3				Aug. 20, 1938 Jan. 6, 1939	6 6	1.39 ± 0.09 1.25 ± 0.11	Aug. 17, 1938 Jan. 5, 1939	7 7	2.73 ± 0.05 2.48 ± 0.02

TABLE 14.
Measurements of various parts in Group I, V, and VI (cm.).

Group	Date of measurements	No. of obs.	L	A	B	C	D	E
I	July 1, 1936	30	0.720 ± 0.004	0.087 ± 0.001	0.090 ± 0.001	$C' \quad 0.104 \pm 0.002$ $C' + D' \quad 0.345 \pm 0.003$	$D' \quad 0.241 \pm 0.002$	0.197 ± 0.002
V	Oct. 6, 1936	25	16.65 ± 0.13	2.40 ± 0.02	2.12 ± 0.03	2.84 ± 0.04	4.47 ± 0.05	4.52 ± 0.05
VI	Aug. 19, 1938 Jan. 7, 1939	17 9	6.29 ± 0.28 7.22 ± 0.44	1.08 ± 0.04 1.23 ± 0.07	0.85 ± 0.04 0.98 ± 0.06	1.14 ± 0.06 1.29 ± 0.08	1.52 ± 0.07 1.61 ± 0.11	1.69 ± 0.08 2.10 ± 0.13
			P	Q	R	X	Y	Z
I	July 1, 1936	30	0.124 ± 0.001	0.090 ± 0.002	0.059 ± 0.001			
V	Oct. 6, 1936	25	3.98 ± 0.04	5.12 ± 0.045	3.71 ± 0.04	2.94 ± 0.03	2.67 ± 0.03	1.50 ± 0.02
VI	Aug. 19, 1938 Jan. 7, 1939	17 9	1.49 ± 0.06 1.63 ± 0.09	2.00 ± 0.10 2.07 ± 0.11	1.36 ± 0.07 1.46 ± 0.08	1.20 ± 0.05 1.31 ± 0.07	1.09 ± 0.05 1.12 ± 0.07	0.57 ± 0.03 0.49 ± 0.03

III. ABSOLUTE GROWTH.

1. General.

When the average values of each measurement are plotted against age, the values of Group I being the initial point, although the date of hatching was 5 days previously, the growth-curves comparing the course of growth in each group can be obtained (Fig. 2).

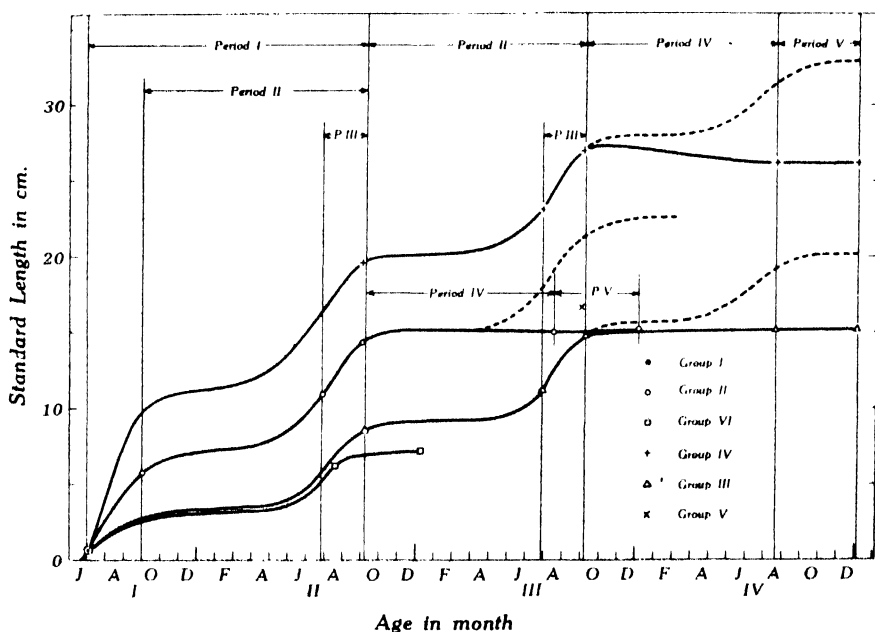


Fig. 2. Growth curve of the standard length.

The conditions of the medium may be considered to be the same in each group without exception, for the water was always running in the same condition and the water-source was the same, and the changes of water-temperature had almost a similar trend during the course of this investigation. The average water-temperatures are as shown in Table 15 (Fig. 3).

In certain fish, such as plaice and haddock, THOMPSON (1917) and others have found clear evidence that the ascending curve of growth is subject to seasonal interruptions, the rate during the winter months always being slower than in the summer months.

He has described this fact as "It is as though we superimposed a periodic, annual, sine-curve upon the continuous curve of growth". And further he says that "as growth itself grows less and less from year to year, so will the difference between the winter and the summer rate also grow less and less. The fluctuation in rate will represent a vibration which is gradually dying out: the amplitude of the sine curve will gradually diminish till it disappears: in short, our phenomenon is simply expressed by what is known as a 'damped sine-curve'."

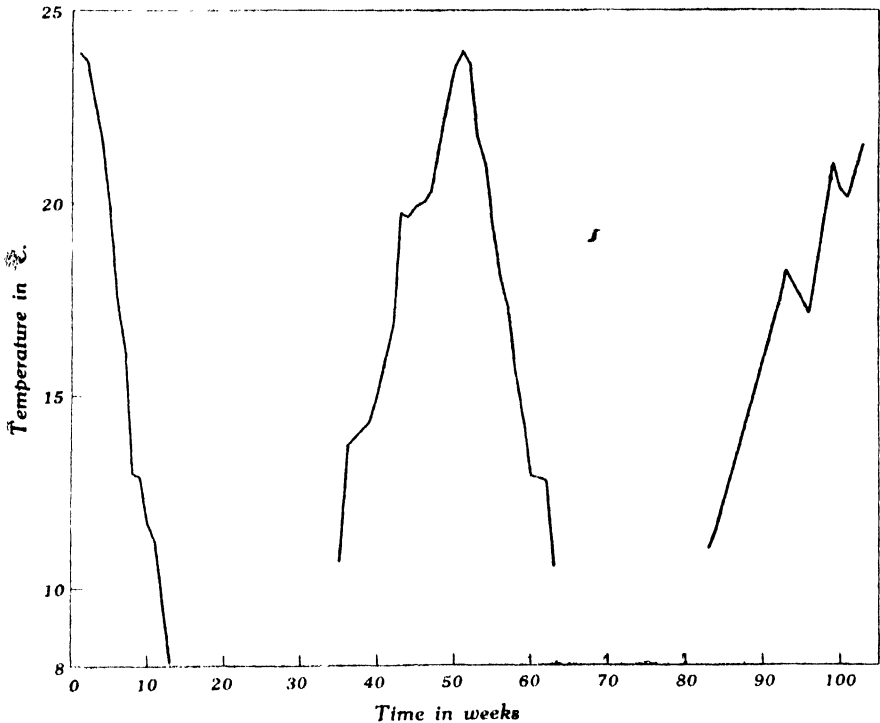


Fig. 3. Average water temperature.

This nature of the growth-curve is the general aspect of growth in poikilothermal animals which experience two years of growth-period or more in their life, when they are exposed to seasonal changes of circumstances. *e. g.* HAMAI (1935) has determined the annually repeated sigmoid curve of growth in the Mollusca, *Meretrix meretrix*, and has proved that the amplitude of this curve grows smaller and smaller from year to year, since the rate of growth in each time-phase gradually de-

TABLE 15.
Weekly Average Temperature (°C) of the Pond-water at 1-2 p.m.

Week	Period of week	Group		Group IV		Group V	Group VI		Average	
		II	III	Pond A	Pond B		Pond A	Pond B	M	σ
1	1936. 1-7/IX	23.9	21.8	22.6		21.5			23.9	1.6
2	8-14	23.7	24.2	23.1		23.7			23.7	0.5
3	15-21	22.8	23.3	21.7		22.8			22.7	1.1
4	22-28	21.7	22.5	20.1		22.5			21.7	1.2
5	29-5/X	20.6	20.6	19.6	19.4	19.9			20.0	1.5
6	6-12	17.1	17.5	17.4	17.0	18.0			17.5	0.7
7	13-19	16.5	16.2	16.2	16.1	16.2			16.2	0.1
8	20-26	12.5	13.6	12.9	12.1	13.8			13.0	1.2
9	27-2/XI	12.4	13.1	12.8	12.6	13.5			12.9	1.1
10	3-9	11.8	11.8	11.6	11.4	12.1			11.7	0.9
11	10-16	10.7	11.3	11.2	11.1	11.6			11.2	0.4
13	21-30	7.0	7.4	8.8	9.0	8.5			8.1	0.8
35	1937 27-3/V	11.1	11.0	10.2	9.6	11.2			10.7	1.5
36	4-10	11.3	15.0	11.9	11.0	16.1			13.7	2.1
39	25-31	15.2	15.6	12.6	12.4	15.8			14.3	2.1
40	1-7/IV	16.6	15.5	13.0	12.7	17.0			15.0	1.8
42	15-21	18.4	18.0	15.0	15.2	17.5			16.8	1.7
43	22-28	20.7	21.2	17.9	18.0	20.8			19.7	1.7
44	29-5/VII	19.8	20.2	19.2	18.9	19.9			19.6	0.9
45	6-12	20.0	19.8	20.0	20.0	19.9			19.9	0.1
46	13-19	20.6	20.6	19.2	19.7	20.1			20.0	0.6
47	20-26	21.0	21.1	19.3	19.8				20.3	0.8
48	27-2/VIII	21.5	23.0	20.9	20.8	21.2			21.5	0.8
49	3-9	23.0	23.0	22.2	22.0				22.6	0.5
50	10-16	23.5	24.1	23.3	23.1				23.5	0.5
51	17-23	23.9	24.2	24.0	23.6				23.9	0.2
52	24-30	23.5	23.6	24.0	23.4				23.6	0.2
53	31-6/IX	21.5	21.5	22.0	22.0				21.7	0.4
54	7-13	20.5	21.0	21.3	21.2				21.0	0.7
55	14-20	19.0	19.1	19.3	19.3		20.1	18.8	19.3	0.5
56	21-27	18.1	18.4	18.0	18.0		18.6	17.8	18.1	0.4
57	28-4/X	17.4	17.5	17.0	16.8		17.6	16.8	17.2	0.7
58	5-11	15.6	15.6	15.6	15.6		15.5	15.5	15.5	0.5
59	12-18	14.1	14.7	14.3	14.0		14.7	14.0	14.3	1.2
60	19-25	12.5	13.9	12.4	12.2		14.0	12.4	12.9	0.7
62	2-8/XI	12.9	13.4	12.4	12.3		13.5	12.4	12.8	0.7
63	9-15	10.9	10.6	10.5	10.5		10.7	10.5	10.6	0.1
83	1938. 29-4/IV	11.0	11.0	11.0	11.0		11.0	11.0	11.0	0.0
84	5-11	11.0	10.1	12.0	13.0		10.9	12.0	11.5	0.9
92	31-6/VI	17.6	18.0	17.6	17.1		17.2	17.0	17.4	1.0
93	7-13	18.0	18.8	19.0	17.6		18.0	17.8	18.2	0.5
96	28-4/VII	17.0	16.5	18.0	17.5		16.5		17.1	0.6
99	19-25	20.6	21.6	20.2	22.0		20.4		21.0	0.7
100	26-1/VIII	20.2	21.3	19.8	20.4		20.0		20.3	0.5
101	2-8	20.0	20.2	20.0	20.5		20.0		20.1	0.2
103	16-22	21.6	21.9	21.0	21.0		22.5		21.5	1.2

creases from year to year. Each cycle of growth is subject to a kind of logistic curve.

In the present case of *Cyprinus carpio*, such an aspect is clearly recognizable, and is expressed as a repeated sigmoid curve similar to a 'damped sine-curve' (Fig. 2).

Group II includes the specimens born in the summer of 1936, and Groups III and IV are those born in the summer of 1935. Comparison of the growth-data of Groups II and III-IV elucidates the differences of growth affected by the age-factor, and the differences between Groups III and IV provide an illustration of the evidence that the growth-rate is affected by the size-factor.

2. Effect of Age on Growth.

From the initial point of growth expressed by the data of birth and the size of Group I, to September 29-October 7 in the second year of growth, is defined as Period I; from September 29 October 7, 1936 to October 4-6, 1937 as Period II; and from July 26-27 to October 4-6, 1937 as Period III (Fig. 2). The increases in the various dimensions during each period are tabulated in Tables 16, 17 and 18. Period I is made up of about the first 15 months after hatching, Period II consists of about the 12 months after Period I, and Period III of about the last 10 weeks of Period II, this being the intense growth-period from summer to autumn. The differences of growth between the groups are of considerable magnitude during Period I (Table 19). Converting the growth-rates during each period into an annual rate, assuming that the rates of each period are constant during those periods respectively, the comparable figures of growth-rate may be obtained (Table 20). It is reliable to recognise the diminishing rate from Period I to Period II.

In Group IV, the rate of absolute growth per annum diminished about by half or more in every dimension except the dimension C. In Group III, however, very minute or no decrease of rate is shown in various dimensions. This fact is explicable as the effect of size factor explained later. The decrease of rate in Group II from Period I to II, expresses the gradual decrease during the initial 15 months, *i.e.* from the hatching in the summer season to the end of the first year (the first growth-cycle), the most intensive growth occurs and the diminished rate appears in the second cycle of growth (Table 20, Fig. 4, 5). After about 15 months from hatching the difference of growth between Groups III and IV becomes 11.10 ± 0.34 cm. in standard length, and further after the next 12 months, the difference of 12.25 ± 0.96 cm. appears. Group II is the intermediate size of the above two groups. It is shown in Fig. 4 that the growth-rate of Group II lies between that of Groups III and IV, in the various dimensions. But in the next growth-cycle, *i.e.* in the 12 months after the initial 15 months, the growth rate of the larger group also

TABLE 16.
Increase of various dimensions during about 15 months after hatching (cm.).

Group	Growth period	Dimensions							
		L	A	B	C ¹	D ²	E	P	Q R
II	From hatching ¹ to Oct. 6 in the 2nd year	13.55 ±0.55	1.96 ±0.07	1.74 ±0.08	2.44 ±0.06	3.93 ±0.17	3.48 ±0.19	3.13 ±0.13	4.87 ±0.20 3.34 ±0.14
III	From hatching ¹ to Oct. 7 in the 2nd year	7.84 ±0.24	1.28 ±0.04	1.05 ±0.03	1.45 ±0.07	2.02 ±0.05	2.06 ±0.08	1.93 ±0.06	2.81 ±0.06 2.00 ±0.05
IV	From hatching ¹ to Sept. 29 in the 2nd year	18.94 ±0.24	2.66 ±0.03	2.66 ±0.04	3.19 ±0.07	5.30 ±0.07	5.13 ±0.07	4.38 ±0.04	6.07 ±0.09 4.39 ±0.06

¹ The size of Group I measured 5 days after hatching was taken as that at the time of hatching.

² The values of C and D are approximate, since the dimensions of Group I are different from the other groups as illustrated in Fig. 1, but there is little difference.

TABLE 17.
Increase of various dimensions during the period of one year (cm.).

Group	Growth period	Dimensions									
		L	A	B	C	D	E	P	Q	R	X Y Z
II	From Oct. 1, '36 to Oct. 6, '37	8.43 ±0.57	1.05 ±0.07	1.03 ±0.08	1.47 ±0.07	2.61 ±0.17	2.28 ±0.19	1.72 ±0.14	2.91 ±0.21	2.06 ±0.15	1.49 ±0.12 1.64 ±0.12 0.89 ±0.06
III	From Oct. 7, '36 to Oct. 6, '37	6.20 ±0.92	0.65 ±0.10	0.79 ±0.11	1.24 ±0.17	1.71 ±0.30	1.81 ±0.27	1.25 ±0.18	2.02 ±0.26	1.43 ±0.21	0.99 ±0.16 1.23 ±0.16 0.67 ±0.09
IV	From Sept. 29, '36 to Oct. 4, '37	7.35 ±0.44	0.54 ±0.07	0.59 ±0.06	1.83 ±0.17	2.13 ±0.13	2.25 ±0.12	1.21 ±0.11	2.42 ±0.18	1.82 ±0.13	1.42 ±0.10 1.55 ±0.13 1.39 ±0.05

TABLE 18.
Increase of various dimensions during the period of 10 weeks, during which the absolute growth rate is intense.

Group	Growth period	Dimensions											
		L	A	B	C	D	E	P	Q	R	X	Y	Z
* II	From July 26, '37 to Oct. 6, '37	3.42 ±0.71	0.39 ±0.03	0.39 ±0.11	0.44 ±0.10	1.06 ±0.22	1.14 ±0.23	0.65 ±0.15	0.88 ±0.27	0.75 ±0.18	0.66 ±0.14	0.44 ±0.14	0.40 ±0.07
	From July 27, '37 to Oct. 6, '37	3.56 ±1.05	0.29 ±0.10	0.46 ±0.14	0.65 ±0.20	1.19 ±0.34	0.98 ±0.32	0.82 ±0.20	1.30 ±0.30	0.87 ±0.24	0.65 ±0.18	0.88 ±0.18	0.50 ±0.10
IV	From July 26, '37 to Oct. 4, '37	3.94 ±0.46	0.33 ±0.07	0.32 ±0.07	0.97 ±0.17	1.27 ±0.16	1.03 ±0.13	0.85 ±0.11	1.13 ±0.19	0.90 ±0.13	1.04 ±0.09	0.77 ±0.13	0.37 ±0.06

TABLE 19.
Differences of growth among Groups II, III, and IV, during about 15 months after hatching.

[illegible]

TABLE 20.

Absolute growth rate per annum. Period I is the initial 15 months, Period II the following year, and Period III the 10 weeks of intense growth in Period II.

Period	Group	L	A	B	C	D	E	P	Q	R	X	Y	Z
I	II	10.8	1.6	1.4	2.0	3.1	2.8	2.5	3.9	2.7			
	III	6.3	1.0	0.8	1.2	1.6	1.6	1.5	2.2	1.6			
	IV	15.2	2.1	2.1	2.6	4.2	4.1	3.5	4.9	3.5			
II	II	8.4	1.1	1.0	1.5	2.6	2.3	1.7	2.9	2.1	1.5	1.6	0.9
	III	6.2	0.7	0.8	1.2	1.7	1.8	1.3	2.0	1.4	1.0	1.2	0.7
	IV	7.4	0.5	0.6	1.8	2.1	2.3	1.2	2.4	1.8	1.4	1.6	1.4
III	II	17.8	2.0	2.0	2.3	5.5	5.9	3.4	4.6	3.9	3.4	2.3	2.1
	III	18.5	1.5	2.4	3.4	6.2	5.1	4.3	6.8	4.5	3.4	4.6	2.6
	IV	20.5	1.7	1.7	5.0	6.6	5.4	4.4	5.9	4.7	5.4	4.0	1.9

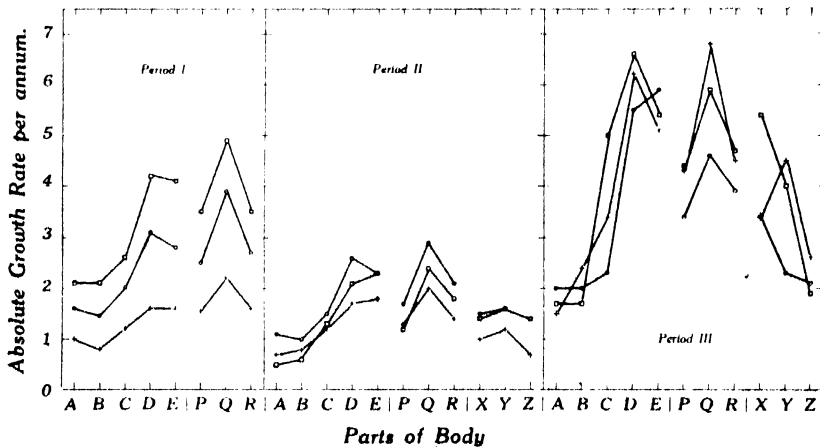


Fig. 4. Absolute growth-rate per annum. ○ Group II, + Group III, □ Group IV.

diminishes to below that of the intermediate group at the initial Period I (Fig. 4). But during Period II, the differences of growth among these three groups are small and lie nearly at the same level as that of the variation of each group. But these results are obtained from the observation of a small number only. The observation of a greater number will show a small but clear difference statistically. At any rate, it can be probably said that in the first period of growth from hatching in summer to the end of the next winter, the most intensive growth-rate takes place, and then, after the second period of growth, *viz.* the second

year after hatching, the growth-rate gradually decreases from year to year : as if a hyperbola rapidly were to descend from infinity to a definite value in the course of a small movement on the abscissa, and thereafter, the decrease of the ordinate to slow down. Such a tendency of the diminishing rate is a general, biological law of dimensional growth in the animal world, and is affected by the age-factor.

In the course of growth the increase of the variation of size has been observed from 4.5 ± 0.4 to 82.1 ± 6.6 in the value of the coefficient of variation during the initial 15 months. This enormous change is, of course, subject to the great individual differences in growth-rate during this period (Period I in Table 20), but its causes are not fully known and should be an interesting subject for study. After this initial period, no great change occurs even if allowing for a small increase.

The period from July 26 to October 6 contains the days of the highest temperature as shown in Table 15 and Fig. 3. The growth-rate in this period is the most intensive. The rates of growth per annum in this period (Period III) are two or three times the yearly average rates, *i. e.* twice as great in Group II and three times as great in Groups III and IV. Although the differences of the rate in Period III in these three groups are very slight, a difference of growth-aspect can be probably recognizable : *i. e.* from the evidences that Group II aged one year shows slightly a greater rate in the annual growth than Groups III and IV aged 2 years, but that in the time of intensive growth the rate of Group II was slightly smaller than the others, it is apparent that the period of growth is greater in Group II of the younger age, than in Groups III and IV of the older age, and consequently Group II grows more per annum in comparison with Groups III and IV, even though its growth-rate, in the period of intensive growth, is lower than the others.

It is also observable that both Groups III and IV, aged two years, resemble each other as regards growth, though both the increase in the year and the rates in the period of most intensive growth are greater in Group IV than in Group III.

All the above observations are regarding the standard length, the sum of the dimensions *A*, *B*, *C*, *D*, and *E*. With regard to these various dimensions, there are some variations in growth relations. The differences of growth among these dimensions cause the differences of the relative growth of parts.

3. Effect of Size on Growth.

The comparisons between Groups III and IV, both of the same age, give some evidence regarding the effect of size. It is clear that the growth of the various dimensions namely the standard length, the sum of these dimensions, are intenser in Group IV of greater size than in Group III of smaller size, in the initial 15 months. It is also true that the group attaining to a higher rate grew larger during this period (Fig. 4). In the next period, the other conditions being all the same, which group will attain to a greater rate is in question. In period II, the anterior

parts (head-part) gained a higher rate in the smaller sized group than in the larger, both in the direction of antero-posterior axis and in the dorso-ventral direction (depth), but in the posterior part of the body the differences of growth relation between the two groups are inverse. In the dextro-sinistral direction (width) the greater sized group showed a higher rate than the smaller sized group, in the three points actually measured (Fig. 4). As a whole, the standard length, *viz.* the sum of 5 parts, grew slightly larger in the greater sized group than in the smaller.

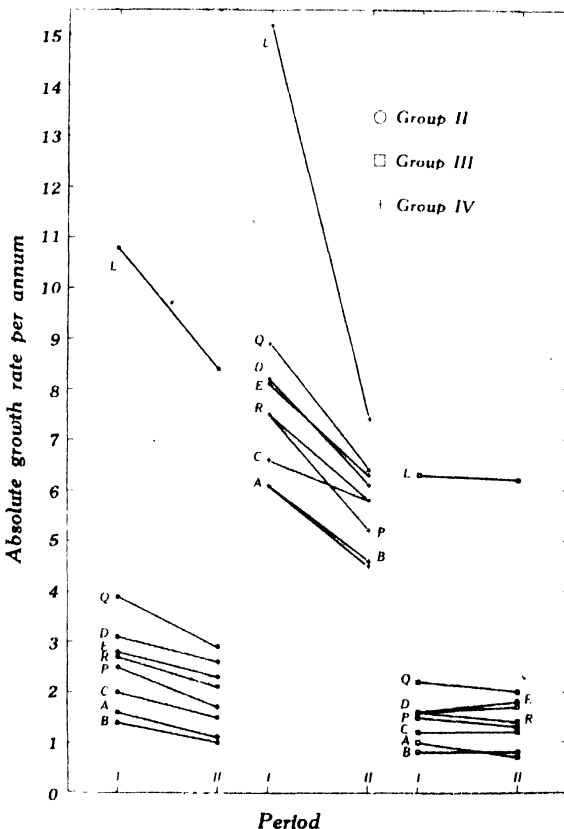


Fig. 5. Decrease of absolute growth-rate in various dimensions from Period I to Period II.

The decrease of the growth rate is more marked in the greater sized group than in the smaller: in the former the rate diminishes about by half, while the latter shows almost no diminution (Table 20, Fig. 5).

In the period of rapid growth, *viz.* in Period III, also the larger sized group showed a larger absolute increase than the smaller, in the total length. Observing the length rate in detail, the posterior head-part of the greater sized group has a lower rate of growth than the smaller, but the other parts of the former show a greater rate than the latter. In depth dimensions *P* and *R* have almost the same rate in the two groups, the dimension *Q* only having a higher rate in the smaller sized group than in the greater. The width increased more at the head part in the larger sized group only (Fig. 4).

The differences of growth-aspect between the data for the year and for the period of intensive growth, and between the larger and smaller sized groups, however, are not so great as compared with individual variations. It is, at any rate, difficult to explain the differences in the growth of the various parts by means of the absolute growth-rate. It must be done by the consideration of the problem in relation to the relative growth-rates and their ratios.

4. Effect of Diet on Growth.

From October 7, 1937 till August 20, 1938 (about 10½ months) the diet was diminished by half. During this period, it was clearly seen little or no growth took place in each group (Table 3-14). Further, no diet was given to the groups after August 20, 1938 until January 6, 1939, in which period normally, when on full diet, half the growth of the year would appear. The measurements on January 6, 1939 showed no growth, or rather a little decrease in the dimension *Z*, especially in Group IV. The head-part is mainly composed of the skeletal structure, and part of trunk contains the visceral cavity, the contents of which influence its dimensions. Therefore the tail-part is the most acceptable indicator for positive or negative growth. Dimensions *R* and *Z* are, accordingly, good indicators of the growth becoming thick or thin. During the period of this small and no diet, negative growth occurred, so little was it. Half diet does not cause any growth and only maintains the body with no increase or decrease. A smaller amount than this seems to cause negative growth.

IV. INDIVIDUAL GROWTH.

1. General.

Some individuals were distinguished by remarkable body characteristics, *e. g.* colour; shape of the fins, of the scales, and of the operculum. These especially prominent characteristics were the marks of individuals distinguish-

ing them from the other specimens. These marks did not change during this investigation. These are, however, not abnormal, but only variations special to the individuals, and have nothing to do with the object of this investigation.

In all, 12 specimens were selected, in which 3 (No. 1-3) belonged to Group II, 5 (No. 4-8) to Group III, and 4 (No. 9-12) to Group IV. Generally speaking, each specimen grew according to the average results mentioned in the previous chapter. From September-October, 1936 to October, 1937, *viz.* during about one year of full diet, a growth-cycle showing a sigmoid curve was formed by every specimen. But in the

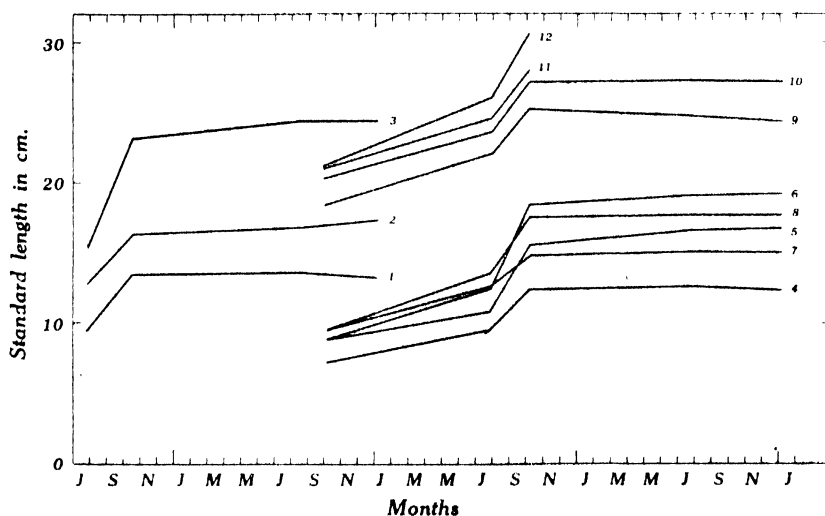


Fig. 6. Individual growth-curve of the standard length. The figures numbered near the curve are the number of specimens.

period of small or no diet, from October, 1937 to August, 1939, little or no growth occurs in every specimen. The measurements of various dimensions are shown in Table 21, and the growths of the standard length are figured in Fig. 6.

2. Absolute Growth.

The specimens, Nos. 1 and 2, of Group II grew by 3-4 cms. in standard length in the rapidly growing period from July to October, while No. 3 grew about 8 cms. in the same period, and this growth is twice that of the others. This giant-like specimen attained a size above the mean of Group IV, which is not only one year older than Group II, but is the largest of its year in size, the measurements being taken in October

TABLE 21.
Measurements of selected specimens.

No.	Group	Date	L	A	B	C	D	E	P	Q	R	X	Y	Z
1	II	July 26, 1937	9.5	1.5	1.2	1.8	2.8	2.2	2.6	3.6	2.3	2.0	2.1	0.9
		Oct. 6, 1937	13.5	1.8	1.7	2.6	3.9	3.5	3.2	4.7	3.2	2.7	2.8	1.3
		Aug. 18, 1938	13.6	1.9	1.8	2.7	3.6	3.6	3.4	4.3	3.1	2.7	2.5	1.3
		Jan. 5, 1939	13.3	2.0	1.8	2.6	3.3	3.6	3.1	4.1	2.9	2.5	2.3	1.2
2	II	July 26, 1937	12.9	1.9	1.8	2.3	3.7	3.2	3.0	4.9	3.2	2.4	2.7	1.2
		Oct. 6, 1937	16.3	2.3	2.2	2.7	4.6	4.5	3.7	5.7	3.9	3.1	3.1	1.6
		Aug. 18, 1938	16.9	2.3	2.5	3.0	4.8	4.3	3.7	5.5	4.0	3.0	3.1	1.8
		Jan. 5, 1939	17.4	2.5	2.4	3.4	4.6	4.5	3.9	5.2	3.8	3.3	2.8	1.7
3	II	Oct. 1, 1936	7.9	1.4	1.1	1.3	2.3	1.8	2.7	3.0	1.8	1.8	1.7	0.8
		July 26, 1937	15.4	2.3	2.0	2.9	4.5	3.7	3.7	6.1	4.1	3.2	3.6	1.6
		Oct. 6, 1937	23.2	3.2	3.1	3.8	6.8	6.3	5.1	8.3	5.8	4.6	4.7	2.2
		Aug. 18, 1938	24.4	3.5	3.4	4.3	6.7	6.5	5.7	7.8	5.6	4.8	4.5	2.6
4	III	Jan. 5, 1939	24.5	3.5	3.6	3.6	7.0	6.8	5.5	7.4	5.5	4.9	4.5	2.4
		Oct. 7, 1936	7.2	1.2	1.0	1.2	1.9	1.9	1.7	2.5	1.8	1.4	1.3	0.7
		July 27, 1937	9.5	1.6	1.3	1.6	2.2	2.8	2.1	3.2	2.2	1.7	1.6	0.9
		Oct. 6, 1937	12.5	1.8	1.6	2.3	3.1	3.7	2.8	4.2	3.0	2.1	2.4	1.3
5	III	Aug. 20, 1938	12.6	1.9	1.7	2.0	3.3	3.7	2.9	3.8	2.7	2.2	2.0	1.1
		Jan. 6, 1939	12.3	2.0	1.8	1.9	3.0	3.6	2.7	3.5	2.5	2.1	1.6	0.8
		Oct. 7, 1936	8.8	1.4	1.2	1.5	2.4	2.3	2.1	3.0	2.1	1.7	1.6	0.9
		July 27, 1937	10.9	1.8	1.4	2.0	2.8	2.9	2.5	3.5	2.5	2.0	1.9	1.0
6	III	Oct. 6, 1937	15.6	2.1	2.2	2.8	4.1	4.4	3.4	5.2	3.6	2.9	2.9	1.6
		Aug. 20, 1938	16.6	2.4	2.4	2.7	4.4	4.7	3.7	5.0	3.5	3.0	2.7	1.4
		Jan. 6, 1939	16.7	2.4	2.5	2.7	4.2	4.9	3.5	4.4	3.5	3.0	2.5	1.3
		Oct. 7, 1936	8.8	1.2	1.1	1.7	2.4	2.4	2.1	2.8	2.1	1.7	1.7	0.9
7	III	July 27, 1937	12.5	1.7	1.6	2.5	3.3	3.4	2.7	3.8	2.8	2.1	2.2	1.3
		Oct. 6, 1937	18.4	2.3	2.4	3.4	5.5	4.8	3.9	6.1	4.3	3.4	3.7	2.0
		Aug. 20, 1938	19.1	2.4	2.7	3.6	5.0	5.4	4.0	5.4	4.0	3.2	3.2	1.8
		Jan. 6, 1939	19.2	2.4	2.7	3.2	5.4	5.5	4.0	5.1	4.0	3.3	3.0	1.8
8	III	Oct. 7, 1936	9.3	1.5	1.2	1.8	2.2	2.6	2.2	3.1	2.2	1.6	1.5	1.0
		July 27, 1937	12.6	1.9	1.6	2.5	3.2	3.4	2.7	4.0	3.0	2.3	2.3	1.2
		Oct. 6, 1937	14.8	2.1	1.8	2.8	4.1	4.0	3.3	4.9	3.5	2.6	2.9	1.6
		Aug. 20, 1938	15.1	2.2	2.1	2.8	4.0	4.0	3.3	4.6	3.3	2.7	2.7	1.4
9	IV	Jan. 6, 1939	15.0	2.3	2.0	2.7	3.9	4.1	3.3	4.4	3.1	2.8	2.5	1.4
		Oct. 7, 1936	9.4	1.6	1.1	1.9	2.3	2.5	2.3	3.0	2.2	1.8	1.7	1.0
		July 27, 1937	13.5	1.9	1.8	2.7	3.2	3.9	2.9	4.4	3.3	2.2	2.2	1.2
		Oct. 6, 1937	17.5	2.3	2.2	3.4	4.5	5.1	4.0	5.6	4.2	2.8	2.9	1.8
10	IV	Aug. 20, 1938	17.8	2.4	2.3	3.1	4.5	5.5	3.9	5.4	3.9	3.0	2.8	1.7
		Jan. 6, 1939	17.7	2.4	2.4	3.1	4.6	5.2	3.7	5.0	3.8	2.9	2.6	1.4
		Sept. 29, 1936	18.4	2.7	2.6	2.9	5.5	4.7	4.4	6.2	4.3	3.5	3.6	1.3
		July 26, 1937	22.1	2.8	3.1	3.8	5.7	6.7	4.8	6.8	4.7	3.5	3.8	2.2
11	IV	Oct. 4, 1937	25.3	3.2	3.2	4.8	6.9	7.2	5.2	8.0	5.5	4.6	4.6	2.6
		Aug. 17, 1938	24.9	2.9	3.3	4.6	6.6	7.5	5.2	6.9	1.9	4.3	4.0	2.6
		Jan. 5, 1939	24.5	2.9	3.4	4.2	6.7	7.3	5.5	6.6	5.0	4.2	3.8	2.3
		Sept. 29, 1936	20.3	2.7	2.9	3.3	5.9	5.5	4.5	6.1	4.5	3.6	3.7	1.3
12	IV	July 26, 1937	23.6	3.1	3.1	3.9	6.6	6.9	4.7	7.3	5.4	4.0	4.6	2.5
		Oct. 4, 1937	27.3	3.2	3.2	5.3	7.6	8.0	6.0	9.3	6.6	5.1	5.2	3.0
		Aug. 17, 1938	27.3	3.1	3.1	5.1	7.9	8.1	5.6	8.2	6.0	4.5	4.7	2.6
		Jan. 5, 1939	27.2	3.2	3.4	5.0	7.5	8.1	5.6	7.9	6.0	4.5	4.6	2.5
13	IV	Sept. 29, 1936	21.0	2.7	2.9	4.0	5.8	5.6	4.6	6.3	4.8	3.9	4.0	1.6
		July 26, 1937	24.6	3.0	3.3	4.6	7.1	6.6	5.0	7.8	5.7	4.0	4.9	2.4
		Oct. 4, 1937	28.0	3.1	3.7	5.6	8.1	7.5	5.8	8.3	6.4	4.8	5.1	2.8
		Sept. 29, 1936	21.2	3.0	3.0	3.6	5.9	5.7	4.8	6.5	4.8	4.0	4.3	1.5
14	IV	July 26, 1937	26.1	3.3	3.4	4.6	7.5	7.3	5.5	8.4	6.1	4.5	4.9	2.4
		Oct. 4, 1937	30.6	3.7	3.6	6.5	8.4	8.4	6.7	10.2	7.1	5.5	6.2	3.3

about 15 months after hatching. The giant was then eliminated from the mean of his group. This great increase is nearly equal to one-year's increase of other fishes. The growth of this specimen during a year was also nearly twofold or more that of the others. An individual, attaining to a larger size than the others at a young stage of growth, becomes a superior in acquiring food. In fact, when the food is thrown into the pond, the largest fish eats them first, competing against all other fishes. He would be able to obtain the greatest amount of food, even though the diet was fully given to all enough and to spare.

The specimen, No. 6, in Group III, was of an intermediate size in October, 1936, and thereafter, in October, 1937 after about one year, it grew to be the largest of the 5 specimens. No. 7. was at first a larger specimen, but at the end of this period, it became a smaller one. No. 6. showed the most intensive growth during the rapidly growing period from July to October, and also during the one year of this period, and Nos. 4 and 7 showed the reverse relation, having a small rate of growth throughout the year.

In Group IV. the specimens, having a larger rate growth during the rapidly growing period, showed a big growth throughout the year. But, the size of the fish did not determine the growth as in the case of Group III.

In general, in all the specimens, the greater the growth-rate during the rapidly growing period, the greater is the increase during the year including this period: *i. e.* the growth-curves of every individual are all of the same form (Fig. 6). The above fact was found in the growth when on full diet. When the diet was diminished by half, the above relation was also examined. In this case, only the increase, consequently the growth-rate, was reduced in proportion to the increase or the growth-rate in the period of full diet. In the period of no diet, it seemed that every fish ceases to grow. The individual data on growth are tabulated in Tables 21 and 22.

The above facts have been exactly examined by the correlation coefficient between the increases of various periods, and by the correlation coefficient between the size and the increase from this size (Tables 23 and 24). In standard length, favourably high positive correlation-coefficients were found between the growth in the intensive growth-period and the growth in the year including that period, and between the growth in the intensive growth-period when on full diet and the growth in the period of half-diet. That is, in standard length, individuals, showing a great increase during the period of intense growth, would show also a

TABLE 22.
The increase of various dimensions.

Group	No.	Growth of standard length			Growth of Q			Growth of R			Growth of Y			Growth of Z		
		Full diet	Half diet	No diet	Full diet	Half diet	No diet	Full diet	Half diet	No diet	Full diet	Half diet	No diet	Full diet	Half diet	No diet
		Rapidly growing period, from July to Oct.	One year period from Oct. to Oct.	From Oct. to Aug. in the next year.	From Oct. to Aug. in the next year.	From Oct. to Aug. in the next year.	From Oct. to Aug. in the next year.	Rapidly growing period from July to Oct.	One year period from July to Oct.	From Oct. to Aug. in the next year.	Rapidly growing period from July to Oct.	One year period from July to Oct.	From Oct. to Aug. in the next year.	Rapidly growing period from July to Oct.	One year period from July to Oct.	From Oct. to Aug. in the next year.
II	1	4.0		0.1-0.3	1.1	-0.4-0.2	0.9	-0.1-0.2	0.7	-0.3-0.2	0.4	0.0	-0.1	0.4	0.0	-0.1
	2	3.4		0.6 0.5	0.8	-0.2-0.3	0.7	0.1-0.2	0.4	0.0-0.3	0.4	0.2	-0.1	0.4	0.2	-0.1
	3	7.8	15.3	1.2 0.1	2.2	-0.5-0.4	1.7	-0.2-0.1	1.1	3.0 -0.2	0.0	0.6	1.4	0.6	1.4	0.4 -0.2
III	4	3.0	5.3	0.1-0.3	1.0	1.7 -0.4-0.3	0.8	1.2 -0.3-0.2	0.8	1.1 -0.4-0.4	0.4	0.6	-0.2-0.3	0.4	0.6	-0.2-0.3
	5	4.7	6.8	1.0 0.1	1.7	2.2 -0.2-0.6	1.1	1.5 -0.1 0.0	1.0	1.3 -0.2-0.2	0.6	0.7	-0.2-0.1	0.6	0.7	-0.2-0.1
	6	5.9	9.6	0.7 0.1	2.3	3.3 -0.7-0.3	1.5	2.2 -0.3 0.0	1.5	2.0 -0.5-0.2	0.7	1.1	-0.2 0.0	0.7	1.1	-0.2 0.0
	7	2.2	5.5	0.3-0.1	0.9	1.8 -0.3-0.2	0.5	1.3 -0.2-0.2	0.6	1.4 -0.2-0.2	0.4	0.6	-0.2 0.0	0.4	0.6	-0.2 0.0
	8	4.0	8.0	0.3-0.1	1.2	2.6 -0.2-0.4	0.9	2.0 -0.3-0.1	0.7	1.2 -0.1-0.2	0.6	0.8	-0.1-0.3	0.6	0.8	-0.1-0.3
IV	9	3.2	6.9	-0.4-0.4	1.2	1.8 -1.1-0.3	0.8	1.2 -0.6 0.1	0.8	1.0 -0.6-0.2	0.4	1.3	0.0-0.3	0.4	1.3	0.0-0.3
	10	3.7	7.0	0.0-0.1	2.0	3.2 -1.1-0.3	1.2	2.1 -0.6 0.0	0.6	1.5 -0.5-0.1	0.5	1.7	-0.4-0.1	0.5	1.7	-0.4-0.1
	11	3.4	7.0		0.5	2.0	0.9	1.6	0.9	1.1	0.4	1.2		0.4	1.2	
	12	4.5	9.4		1.8	3.7	1.3	2.3	0.6	1.9	0.9	1.8		0.9	1.8	

high growth, even when the diet was diminished by half. The depths of the fish, *viz.* dimensions Q and R , show an intensive growth throughout the year, growing mostly in the rapidly growing period of that year. In the widths, *viz.* the dimensions Y and Z , no probable correlation was found between these growths.

TABLE 23.*
Correlation coefficient between the intensive growths and the growths during the other periods.

	Dimensions	Full diet, One year period	Half diet	No diet
Full diet Rapidly growing period	Standard length	0.96 ± 0.02	0.73 ± 0.10	0.33 ± 0.19
	Q	0.73 ± 0.10	-0.42 ± 0.18	-0.38 ± 0.18
	R	0.83 ± 0.07	-0.14 ± 0.21	0.36 ± 0.19
	Y	0.42 ± 0.18	-0.38 ± 0.18	0.27 ± 0.20
	Z	0.46 ± 0.17	-0.04 ± 0.21	0.16 ± 0.21
	PX	0.86 ± 0.02	-0.24 ± 0.20	0.06 ± 0.21
	QY	0.89 ± 0.04	-0.68 ± 0.12	-0.12 ± 0.21
	RZ	0.94 ± 0.03	-0.25 ± 0.20	-0.17 ± 0.21

TABLE 24.*
Correlation coefficient between the size and the growth from that size.

	Rapidly growing period from July to Oct., 1937	One year period from Sept.-Oct., 1936 to Oct., 1937	From Oct., '37 to Aug., 1938	From Aug., '38 to Jan., 1936
Standard length	-0.17 ± 0.19	-0.48 ± 0.17	-0.16 ± 0.21	0.00 ± 0.21
Q	0.09 ± 0.19	0.04 ± 0.21	-0.77 ± 0.09	-0.16 ± 0.21
R	0.25 ± 0.18	0.00 ± 0.21	-0.65 ± 0.12	0.42 ± 0.18
Y	-0.17 ± 0.19	-0.16 ± 0.21	-0.49 ± 0.16	0.78 ± 0.08
Z	0.18 ± 0.19	0.63 ± 0.13	-0.13 ± 0.21	-0.17 ± 0.21
PX	0.56 ± 0.13	0.51 ± 0.16	-0.47 ± 0.17	0.19 ± 0.21
QY	0.52 ± 0.14	0.49 ± 0.16	-0.84 ± 0.06	-0.25 ± 0.20
RZ	0.75 ± 0.09	0.73 ± 0.10	-0.50 ± 0.16	-0.39 ± 0.18

* The probability, that a correlation should arise, by random sampling, from an uncorrelated population, is 0.05 when $r=0.6319$ and 0.02 when $r=0.7155$ in the cases of the period of one year on full diet and of the periods on half and no diet, and it is 0.05 when $r=0.5760$ and 0.02 when $r=0.6581$ in the case of the period of rapid growth, in Tables 23, 24, 28 and 29 (FISHER, 1936).

TABLE 25.

The relative area sectioned at 3 parts.

No.	Date	PX	QY	RZ
1	July 26, 1937	5.20	7.56	2.07
	Oct. 6, 1937	8.64	13.16	4.16
	Aug. 18, 1938	9.18	10.75	4.03
	Jan. 5, 1939	7.75	9.43	3.48
2	July 26, 1937	7.20	13.23	3.84
	Oct. 6, 1937	11.47	17.67	6.24
	Aug. 18, 1938	11.10	17.05	7.20
	Jan. 5, 1939	12.87	14.56	6.46
3	Oct. 1, 1936	4.86	5.10	1.44
	July 26, 1937	11.84	21.96	6.56
	Oct. 6, 1937	23.46	39.01	12.76
	Aug. 5, 1938	27.36	35.10	14.56
4	Jan. 5, 1939	26.95	33.30	13.20
	Oct. 7, 1936	2.38	3.25	1.26
	July 27, 1937	3.57	5.12	1.98
	Oct. 6, 1937	5.88	10.08	3.90
5	Aug. 20, 1938	6.38	7.60	2.97
	Jan. 6, 1939	5.67	5.60	2.00
	Oct. 7, 1936	3.57	4.80	1.89
	July 27, 1937	5.00	6.65	2.50
6	Oct. 6, 1937	9.86	15.08	5.76
	Aug. 20, 1938	11.10	13.50	4.90
	Jan. 6, 1939	10.50	11.00	4.55
	Oct. 7, 1936	3.57	4.76	1.89
7	July 27, 1937	5.67	8.36	3.64
	Oct. 6, 1937	13.26	22.57	8.60
	Aug. 20, 1938	12.80	17.28	7.20
	Jan. 6, 1939	13.20	15.30	7.20
8	Oct. 7, 1936	3.52	4.65	2.20
	July 27, 1937	6.21	9.20	3.60
	Oct. 6, 1937	8.58	14.21	5.60
	Aug. 20, 1938	8.91	12.42	4.62
9	Jan. 6, 1939	9.24	11.00	4.34
	Oct. 7, 1936	4.14	5.10	2.20
	July 27, 1937	6.38	9.68	3.96
	Oct. 6, 1937	11.20	16.24	7.56
10	Aug. 20, 1938	11.70	15.12	6.63
	Jan. 6, 1939	10.73	13.00	5.32
	Sept. 29, 1936	15.40	22.32	5.59
	July 26, 1937	16.80	25.84	10.34
11	Oct. 4, 1937	23.92	36.80	14.30
	Aug. 17, 1938	22.36	27.60	12.74
	Jan. 5, 1939	23.10	25.08	11.50
	Sept. 29, 1936	16.20	22.57	5.85
12	July 26, 1937	18.80	33.58	13.50
	Oct. 4, 1937	30.60	48.36	19.80
	Aug. 17, 1938	25.20	38.54	15.60
	Jan. 5, 1939	25.20	36.34	15.00
13	Sept. 29, 1936	17.94	25.20	7.68
	July 26, 1937	20.00	38.22	13.68
	Oct. 4, 1937	27.84	42.33	17.92
	Sept. 29, 1936	19.20	27.95	7.20
14	July 26, 1937	24.75	41.16	14.64
	Oct. 4, 1937	36.85	63.24	23.43

Supposing that the products of P and X , of Q and Y , and of R and Z show the relative thickness of the respective parts of the body, these products being proportional to the actual sectioned area of respective body parts, they may be a better index for growth (Tables 25 and 26). In these products, a very high correlation exists between the increase of the period of intensive growth and the increase during one year.

TABLE 26.

The increase of the sectioned area at 3 parts.

No.	Growth of PX				Growth of QY				Growth of RY			
	Full diet		Half diet	No diet	Full diet		Half diet	No diet	Full diet		Half diet	No diet
	Rapidly growing period from July to Oct.	One year period from Oct. to Oct.	From Oct. to Aug.	From Aug. to Jan.	Rapidly growing period from July to Oct.	One year period from Oct. to Oct.	From Oct. to Aug.	From Aug. to Jan.	Rapidly growing period from July to Oct.	One year period from Oct. to Oct.	From Oct. to Aug.	From Aug. to Jan.
1	3.44		0.54	-1.43	5.60		-2.41	-1.32	2.09		-0.13	-0.55
2	4.27		-0.37	1.77	4.44		-0.62	-2.49	2.40		0.96	-0.74
3	11.62	18.60	3.90	-0.41	17.05	33.91	-3.91	-1.80	6.20	11.32	1.80	-1.36
4	2.31	3.50	0.50	-0.71	4.96	6.83	-2.48	-2.00	1.92	2.64	-0.93	-0.97
5	4.86	6.29	1.24	-0.60	8.43	10.28	-1.58	-2.50	3.26	3.87	-0.86	-0.35
6	7.59	9.69	-0.46	-0.40	14.21	17.81	-5.29	-1.98	4.96	6.71	-1.40	0.00
7	2.37	5.06	0.33	0.33	5.01	9.56	-1.79	-1.42	2.00	3.40	-0.98	-0.28
8	4.82	7.06	0.50	-1.03	6.56	11.14	-1.12	-2.12	3.60	5.36	-0.93	-1.31
9	7.12	8.52	-1.56	0.74	10.96	14.48	-9.20	-2.52	3.96	8.71	-1.56	-1.24
10	11.80	14.40	-5.40	0.00	14.78	25.79	-9.82	-2.20	6.30	13.95	-4.20	-0.60
11	7.84	9.90			4.11	17.13			4.24	10.24		
12	12.10	17.65			22.08	35.29			8.79	16.23		

In the period of no diet, a definite correlation was not found. With no regard to the growth-rate in the normal growth, a very slight decrease appeared in the linear dimensions and in the thickness of the body.

In the period of full-diet the size at the beginning of the growth-period seems not to determine the growth of the linear dimensions in general. No probable correlation was found between the size of linear dimensions and the growth from this size. But, in RZ , a probable correlation exists. This correlation explains the fact which could not be

measured in the mere linear dimension.

In the period of half-diet, the significant, negative correlation-coefficients exist in Q and QY : *i.e.* while the thickness of the body, especially RZ , grows more or less according to the mass at the beginning of the growth-period, the depth and perhaps the width too or the thickness, especially Q and QY , also decrease more or less according to the initial size in the case of half-diet, or in the case when the food was taken only in mere maintenance of the body, in consequence chiefly of the decrease of the intestinal contents, and also of the tissue-materials *e.g.* musculature.

Now, an interesting fact has been observed in the dimension Y when no diet is given. That is, a probable positive correlation exists between the size of Y and its negative growth which decreased from this size. It means that the negative growth diminishes as great as the size grows.

In conclusion from the data above mentioned, either the growth-rate under normal conditions or the size of the fish determines the dimensional growth, and both are the factors playing a partial rôle in the growth: the growth-rate is a measure of the activity of life, and the size is a condition of life.

3. Relative Growth.

The absolute growth-rate is, as mentioned above, to be partially conditioned by size. The relative growth-rate must, then, be a true measure of growth, which is given by $\frac{1}{x} \frac{dx}{dt}$, where x is a dimension at growth and t is the time. The relative growth-rate may be more thoroughly determined by the correlation with the size. If the absolute growth-rate and the size are in a linear function, a high correlation-coefficient must be shown between the two. However, the significant correlation was only shown in some cases (Table 24), though the number of observations were very few. Then, the relative growth rate was further examined. In this examination, the relative growth-rate was calculated by $\frac{\log x_2 - \log x_1}{t_2 - t_1}$, where x_1 is the initial size at the beginning of a growth-period (t_1), and x_2 is the final size at the end of this period (t_2). The time was measured by the unit of a year. Accordingly, the relative growth-rate per annum was considered (Table 27).

Each individual shows a different, relative growth-rate, and the differences are considerable even in the same group. As the relative growth-rates of Groups III and IV, which are of the same age, are compared when on full diet, those of the greater sized Group IV are

TABLE

Relative growth-rate $(\frac{\log x_2 - \log x_1}{t_2 - t_1})$

Period	Group	No.	L	A	B	C	D
II One year period; from Sept.-Oct., 1936 to Oct., 1937	II	3	1.06	0.82	1.02	1.06	1.07
	III	4	0.55	0.41	0.47	0.65	0.49
		5	0.55	0.41	0.61	0.63	0.54
		6	0.74	0.65	0.78	0.70	0.83
		7	0.47	0.34	0.41	0.44	0.62
		8	0.62	0.36	0.70	0.58	0.67
	IV	9	0.32	0.17	0.21	0.50	0.22
		10	0.29	0.17	0.10	0.47	0.25
		11	0.28	0.14	0.24	0.33	0.33
		12	0.36	0.21	0.18	0.58	0.35
III Rapidly growing period; from July to Oct., 1937	II	1	1.79	0.91	1.77	1.87	1.68
		2	1.19	0.97	1.02	0.81	1.10
		3	2.08	1.67	2.22	1.37	2.09
	III	4	1.41	0.61	1.05	1.87	1.77
		5	1.84	0.79	2.33	1.73	1.96
		6	1.99	1.56	2.09	1.58	2.63
		7	0.83	0.51	0.61	0.59	1.28
		8	1.33	0.98	1.03	1.19	1.76
	IV	9	0.71	0.70	0.16	1.22	1.00
		10	0.76	0.16	0.16	1.60	0.74
		11	0.68	0.17	0.60	1.03	0.69
		12	0.83	0.60	0.30	1.69	0.59
IV Period of half diet; about 10½ months from Oct., 1937 to Aug., 1938	II	1	0.01	0.06	0.07	0.04	-0.09
		2	0.04	0.00	0.15	0.12	0.05
		3	0.06	0.10	0.11	0.14	-0.02
	III	4	0.01	0.06	0.07	-0.16	0.07
		5	0.07	0.15	0.10	-0.04	0.08
		6	0.04	0.05	0.14	0.07	-0.11
		7	0.02	0.05	0.18	0.00	-0.03
		8	0.02	0.05	0.05	-0.11	0.00
	IV	9	-0.02	-0.11	0.03	-0.05	-0.05
		10	0.00	-0.04	0.04	-0.04	-0.04
V Period of no diet; from Aug., 1938 to Jan., 1939	II	1	-0.06	0.13	0.00	-0.10	-0.23
		2	0.08	0.22	-0.11	0.33	-0.11
		3	0.01	0.00	0.15	-0.46	0.12
	III	4	-0.07	0.13	0.15	-0.13	-0.25
		5	0.02	0.00	0.11	0.00	-0.12
		6	0.01	0.00	0.00	-0.31	0.20
		7	-0.02	0.12	-0.13	-0.10	-0.67
		8	-0.01	0.00	0.11	0.00	0.06
	IV	9	-0.04	0.00	0.08	-0.24	0.04
		10	-0.01	0.08	0.24	-0.05	-0.13

27.

of several individuals.

E	P	Q	R	X	Y	Z	PX	QY	RZ
1.24	0.63	1.01	1.15	0.93	1.01	1.00	1.55	2.01	2.15
0.67	0.50	0.52	0.51	0.41	0.62	0.62	0.89	1.14	1.13
0.65	0.48	0.55	0.54	0.54	0.60	0.58	1.02	1.15	1.12
0.70	0.62	0.78	0.72	0.70	0.78	0.80	1.32	1.56	1.52
0.43	0.41	0.46	0.47	0.49	0.66	0.47	0.89	1.12	0.94
0.71	0.56	0.63	0.65	0.44	0.54	0.59	1.00	1.16	1.24
0.42	0.17	0.25	0.24	0.27	0.24	0.68	0.43	0.49	0.93
0.37	0.28	0.42	0.38	0.34	0.34	0.83	0.63	0.75	1.20
0.29	0.23	0.27	0.28	0.21	0.24	0.55	0.43	0.51	0.84
0.38	0.33	0.44	0.39	0.31	0.36	0.78	0.64	0.81	1.16
2.35	1.05	1.35	1.68	1.52	1.46	1.87	2.58	2.81	3.54
1.73	1.06	0.77	1.12	1.30	0.70	1.46	2.36	1.47	2.46
2.70	1.63	1.56	1.76	1.84	1.35	1.62	3.47	2.92	3.38
1.43	1.48	1.40	1.60	1.09	2.09	1.89	2.57	3.48	3.49
2.15	1.58	2.04	1.88	1.91	2.18	2.42	3.49	4.21	4.30
1.77	1.89	2.43	2.20	2.48	2.67	2.22	4.37	5.10	4.45
0.84	1.03	1.04	0.79	0.63	1.19	1.48	1.67	2.22	2.28
1.38	1.65	1.24	1.24	1.24	1.42	2.09	2.90	2.66	3.33
0.38	0.42	0.85	0.82	1.43	1.00	0.87	1.84	1.85	1.69
0.77	1.28	1.25	1.05	1.27	0.64	0.95	2.54	1.90	2.00
0.67	0.77	0.32	0.60	0.95	0.21	0.93	1.73	0.53	1.41
0.73	1.03	1.01	0.79	1.05	1.23	1.66	2.08	2.24	2.46
0.03	0.07	-0.10	-0.04	0.00	-0.13	0.00	0.07	-0.23	-0.04
-0.05	0.00	-0.04	0.03	-0.04	0.00	0.14	-0.04	-0.04	0.17
0.04	0.13	-0.07	-0.04	0.08	-0.05	0.19	0.18	-0.12	0.15
0.00	0.04	-0.12	-0.12	0.05	-0.21	-0.19	0.09	-0.33	-0.31
0.05	0.09	-0.05	-0.03	0.04	-0.08	-0.15	0.13	-0.13	-0.19
0.14	0.03	-0.14	-0.08	0.07	-0.17	-0.12	-0.04	-0.31	-0.20
0.00	0.00	-0.07	-0.07	0.04	-0.08	-0.15	0.04	-0.16	-0.22
0.09	-0.03	-0.04	-0.09	0.08	-0.04	-0.07	0.05	-0.08	-0.15
0.05	0.00	-0.17	-0.13	-0.08	-0.16	0.00	-0.08	-0.33	-0.13
0.01	-0.08	-0.15	-0.11	0.15	-0.12	-0.17	-0.22	-0.26	-0.27
0.00	-0.25	-0.12	-0.18	-0.20	-0.22	-0.21	-0.44	-0.34	-0.38
0.12	0.14	-0.15	-0.13	0.25	0.26	-0.15	0.39	0.41	-0.28
0.12	-0.09	-0.14	-0.05	0.05	0.00	-0.21	-0.04	-0.14	-0.26
-0.07	-0.19	-0.22	-0.20	-0.12	0.59	0.83	-0.31	-0.80	-1.04
0.11	-0.15	-0.34	0.00	0.00	-0.20	-0.20	-0.15	-0.54	-0.19
0.05	0.00	-0.15	0.06	0.08	-0.17	0.00	0.10	-0.32	0.00
0.07	0.00	-0.12	-0.16	0.10	-0.20	0.00	0.10	-0.32	-0.16
-0.15	-0.14	-0.20	-0.07	-0.09	-0.20	-0.51	-0.23	-0.40	-0.58
-0.07	0.15	-0.12	0.05	-0.06	-0.13	-0.32	0.08	-0.25	-0.27
0.00	0.00	-0.10	0.00	0.00	-0.06	-0.10	0.00	-0.15	-0.10

smaller than those of the smaller sized Group III, generally in all dimensions. In exact proof of this fact, the correlation-coefficient was calculated between the size and the relative growth in some dimensions (Table 28). Thus, significant negative values were obtained. That is, the relative growth-rate during one year and also that during the period of intensive growth, decreases with the increase of size under normal conditions, especially in the standard length, *QY*, and in *RZ*. On the other hand, no significant correlation could be detected between the size and the relative growth-rate during the period of half-diet and no diet, except in the case of *QY* on no diet. The correlation in the period of no diet was also seen in the absolute growth-rate of *Y*, the component of *QY*. Accordingly, it may be recognizable that there is generally no correlation of primary order between the size and the relative growth-rate during the period under abnormal conditions, *viz.* in small diet and in no diet, except only *QY*.

TABLE 28.
Correlation coefficient between the size and the relative growth-rate from that size.

Dimensions	Rapidly growing period	One year period of full diet	Period of half diet	Period of no diet
Standard length	-0.70 ± 0.10	-0.76 ± 0.09	-0.27 ± 0.20	0.19 ± 0.21
<i>PX</i>	-0.46 ± 0.15	-0.65 ± 0.12	-0.52 ± 0.15	0.26 ± 0.20
<i>QY</i>	-0.65 ± 0.11	-0.76 ± 0.09	-0.20 ± 0.21	0.78 ± 0.08
<i>RZ</i>	-0.74 ± 0.09	-0.48 ± 0.16	-0.03 ± 0.21	0.43 ± 0.17

In the absolute growth, high correlations exist between the growth during the intensive growth-period and that of the other periods, *viz.* the greater the growth during the intensive growth-period, the greater is the growth throughout the year under normal conditions. In the relative growth-rate, only the standard length and *PX* show this sort of correlation. No dimensions showed any significant correlation between the relative growth-rate under normal conditions and that under abnormal conditions (Table 29). After all, when the materials maintaining the growth are not fully or quite absorbed, it can be probably said that the absolute growth-rate under normal conditions does not determine the negative growth in the starvation period, but the size and relative growth-rate under normal conditions given an inverse effect on negative growth in some parts. The age factor affecting the positive or negative growth was not definitely found from the observations of these individual growths.

TABLE 29.

Correlation coefficient between the relative growth-rate in the intensive growth-period and that in the other periods.

Dimensions	Full diet one year period	Half diet	No diet
Standard length	0.89 ± 0.04	0.54 ± 0.15	0.13 ± 0.21
PX	0.81 ± 0.07	0.23 ± 0.20	0.09 ± 0.21
QY	0.46 ± 0.17	-0.28 ± 0.20	-0.34 ± 0.19
RZ	0.49 ± 0.16	-0.06 ± 0.21	-0.09 ± 0.21
V ₁	0.87 ± 0.05	0.52 ± 0.16	-0.18 ± 0.21
V ₂	0.67 ± 0.12	-0.34 ± 0.19	-0.45 ± 0.17
V ₃	0.69 ± 0.11	0.19 ± 0.21	0.23 ± 0.20

The growth-gradient of every part must be considered from the relative growth-rate of every part. The axial gradient through the parts, which are measured by the dimensions, *A*, *B*, *C*, *D*, and *E*, and by the depths, *P*, *Q*, and *R*, and by the widths, *X*, *Y* and *Z*, and the relative thicknesses, *PX*, *QY*, and *RZ*, show great variation among individual specimens, and therefore it is difficult to recognize any definite relationship except in consultation of the average results, which will be later given. Roughly speaking, most individuals in the course of the third year's growth, *viz.* most specimens of Groups III and IV, have the growth-centre in the body-trunk region: especially in *C*-region in Group IV and in *C-D*-region in Group III, in the growth of length-direction. In the perpendicular direction to the long axis, there exist, in depth, a growth centre at *Q* region in Group IV and at *Q-R*-region in Group III, which is coincident with that of the length-axis. In the width-growth, Group IV has a growth-centre in *R*-region or *Z*-region strikingly, while Group III has it generally in *Q-R* or *Y-Z* region (Table 27).

These details of the existence of the growth-centre are merely an average result during one year. In the course of growth during one year the growth-centre of every individual more or less changes with time or season: *e.g.* in depth some individuals of Group III changed their centre of growth from *Q-R*-region to the head or *P*-region, and also, some of Group IV changed it from *Q*-region to the head; and in width, some of Group IV changed the centre from *Z*-region to *X*-region, in the period of rapid growth.

In the growth of the second year, Group II shows the growth centre in the posterior region: in *E*, *R* and *Z* regions in average of the year,

and also in the rapidly growing period, in which only one individual, No. 3, was observed. But the weak growth region changed and was shown to be at *C*, *Q* and *Y* in the rapidly growing period, while the average result gives its existence as at the head part.

The growth-gradient of the component dimensions may be merely apparent but not fundamental. In considering the growth fundamentally, the volume must be measured.

In *B*-region, the dimensions, *B*, *P* and *X*, are respectively a component of rectangular coordinates; in *C-D*-region, *C+D*, *Q* and *Y* are respective components; and in *D E* region, *D+E*, *R* and *Z* are the components. Thus, in expressing the relative growth-rate of the volume in *V*₁, *V*₂ and *V*₃ regions, the rates of *B*, *P* and *X*; the average rates of *C* and *D*, and the rates of *Q* and *Y*; and the average rate of *D* and *E*, and the rates of *R* and *Z* are respectively taken. The product of these sets of three dimensions can be stated to be proportional to the volume, *i. e.*

$$v = kxyz$$

where *v* is the volume, *x*, *y* and *z* are respective component dimensions, and *k* a constant. Then, the relative growth-rate of the volume is

$$\begin{aligned} \frac{1}{v} \frac{dv}{dt} &= \frac{1}{kxyz} \frac{d}{dt}(kxyz) \\ &= \frac{1}{x} \frac{dx}{dt} + \frac{1}{y} \frac{dy}{dt} + \frac{1}{z} \frac{dz}{dt} \end{aligned}$$

The sum of the three relative growth-rates of a set is equal to the relative growth-rate of the volume of three parts, *viz.* *V*₁, *V*₂ and *V*₃ regions (Table 30).

In the average of a year, the growth-centre exists in the most posterior region (*V*₃) in Group IV, and in the posterior regions, *V*₂ and *V*₃, in Group III, which are both in the course of the third year's growth, and in Group II, which is in the course of the second year's growth, in the most posterior region of the three. But in the rapidly growing period, in which a half the growth of the year takes place, there exists the second growth-centre in the head region in some specimens of Group II, and some of Group III show the growth-centre in the middle region or in the most posterior region, and in Group IV, it appears in the middle region, except in one individual which showed it in the head and the posterior regions. These facts explain the change of the growth-centre according to the season. The posterior region has, however, always a relatively high growth intensity throughout the year, and this relatively high intensity of growth is maintained at all ages (Fig. 7).

TABLE 30.

*The relative growth rate of the volume of the three parts,
 V_1 , V_2 and V_3 .*

Group	No.	Period III Rapidly growing period			Period II One year period			Period IV Period of half diet			Period V Period of no diet		
		V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
II	1	4.34	4.59	5.57				0.14	-0.26	-0.07	-0.45	-0.51	-0.51
	2	3.38	2.43	4.00				0.11	0.05	0.17	0.28	0.22	-0.27
	3	5.69	4.64	5.78	2.58	3.09	3.31	0.32	-0.06	0.16	0.11	-0.31	-0.14
III	4	3.62	5.31	5.09	1.38	1.71	1.71	0.16	-0.38	-0.28	-0.16	-1.00	0.47
	5	5.82	6.07	6.36	1.63	1.74	1.72	0.23	-0.11	-0.11	-0.04	-0.60	-0.21
	6	6.46	7.21	6.62	2.10	2.33	2.29	0.10	-0.33	-0.18	0.08	0.38	0.13
	7	2.27	3.17	4.33	1.31	1.65	1.47	0.22	-0.17	-0.24	-0.03	-0.71	-0.46
	8	3.92	4.14	4.90	1.70	1.80	1.93	0.10	-0.14	-0.11	-0.12	-0.37	-0.63
IV	9	2.01	2.96	2.38	0.65	0.85	1.24	-0.05	-0.38	-0.13	0.17	-0.35	-0.29
	10	2.71	3.06	2.76	0.72	1.12	1.52	-0.19	-0.31	-0.30	0.24	-0.25	-0.17
	11	2.32	1.39	2.21	0.68	0.84	1.14						
	12	2.38	3.38	3.11	0.82	1.27	1.54						

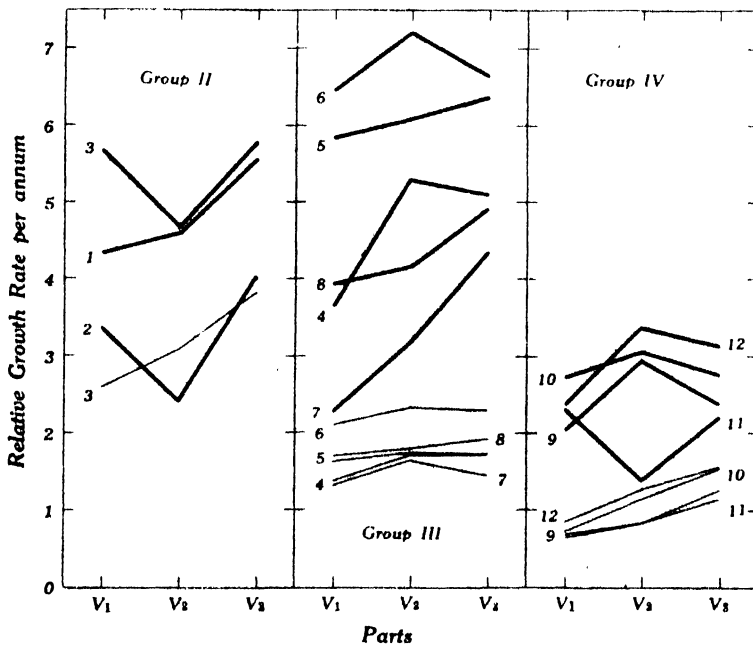


Fig. 7. Growth gradient of individuals. Thick line represents Period III, and thin line Period II. Figures show the number of specimens.

The above descriptions were of the normal conditions when on full diet. In abnormal conditions, the state of growth is different to the normal. At first it is striking that the relative growth-rates of the component dimensions markedly decrease in *C*, *D*, *Q* and *R*, in most specimens of every group when the food is insufficient. In Group III the decreases occurring in *Y* and *Z* are remarkable. But unlike the decreases of the relative growth-rate in the dimensions *C* and *D*, the growth-rate of *A* and *B*, viz. the head part, is relatively rather high compared with the others, and in consequence, the growth-centre seems to remove to the head part. In the case of no diet, the circumstances are almost the same as in the case of small diet. In that case, the variations of the relative growth-rate in every part are very great. On these variations, the error of measurements have a considerable influence, on account of the difficulty of measuring very small negative growths.

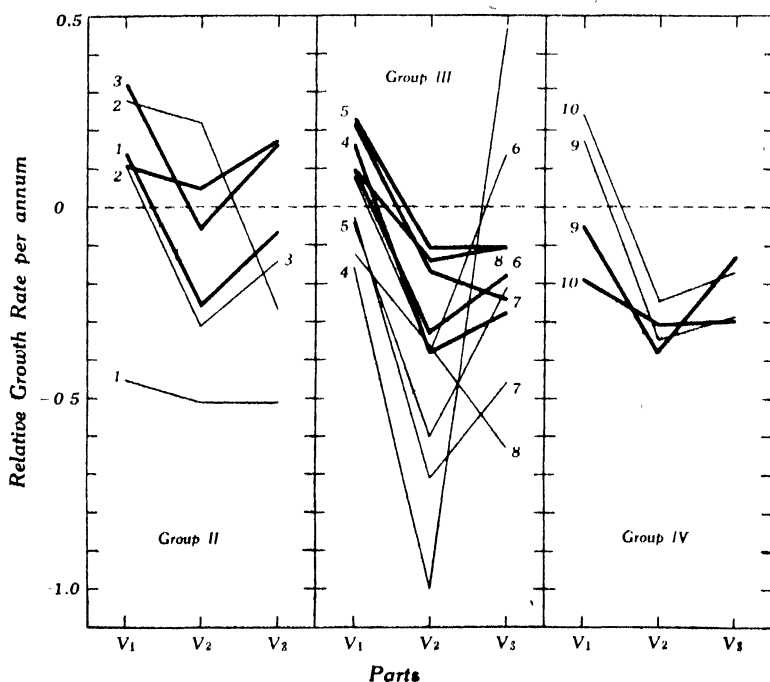


Fig. 8. Growth gradient of several individuals. Thick line shows period of half diet, and thin line period of no diet. Figures show the number of specimens.

On the other hand, when the relative growth-rates of the volume are observed, these various, individual, and apparent growth-gradients are

unified by clear evidence (Fig. 8). That is, in the case of small diet, the growth-centre exists in the anterior region in all groups and in both ages, removing there from the posterior region. But, the relatively high growth-intensities remain in the posterior, in most individuals. This fact shows that the great decrease of the rates occurs in the middle region. This condition appears remarkably in the case of no diet, especially in Group III. In other words, the negative growth-gradient exists in the middle part in most cases, and in the posterior part in a few cases. Roughly speaking, the positive growth-centre under normal conditions becomes a negative one under abnormal conditions. This coincides with a clear decrease in the size of QY .

Between the absolute values of relative growth-rates in the rapidly growing period under normal conditions, and in the other periods under abnormal conditions, no correlation exists significantly, although there is a significant correlation in V_1 between the rapidly growing period and the one year period under normal conditions (Table 29).

In conclusion, the size and the relative growth-rate in the period of intensive growth are the factors conditioning the growth under normal conditions, but they do not condition it under abnormal conditions. The growth-gradient is, however, always definite, and its centre acts positively under normal conditions and negatively under abnormal conditions. Therefore, the relative growth under normal conditions reverses under abnormal conditions.

V. RELATIVE GROWTH.

1. Relative Growth-Rate and its Ratio.

The relative growth rate (R) is defined by $\frac{1}{x} \frac{dx}{dt}$, where x is a growing dimension or mass, and t is the time.

$$R = \frac{1}{x} \frac{dx}{dt} = \frac{d}{dt} \log x$$

Then, when x_1 and x_2 are a measurement at the time t_1 and t_2 respectively lying in a short interval, the relative growth rate can be measured by $\frac{\log x_2 - \log x_1}{t_2 - t_1}$. In the previous chapter, the relative growth rate has already been calculated by this formula from the data of the individual specimens (Table 27). Here, it has been calculated from the average dimensions (Table 31), as the result as a whole cannot be obtained from these meagre data of individual specimens. But it is merely approximate

TABLE 31.

The relative growth-rates of various dimensions at the three periods, calculated from the mean values of the dimensions by means of $\frac{\log x_2 - \log x_1}{t_2 - t_1}$.

Group	Period	L	A	B	C	D	E	P	Q	R	X	Y	Z	V ₁	V ₂	V ₃
II	July 1, '36- Oct. 6, '37	2.36	2.50	2.38	2.52*	2.25*	2.31	2.58	3.17*	3.20	$\left\{ \begin{array}{l} 2.34 \\ C+D \\ 1.90 \\ 2.60 \end{array} \right\}$					
III	July 1, '35- Oct. 7, '36	1.95	2.17	2.00	2.13*	1.76*	1.92	2.21	2.74*	2.81						
IV	July 1, '35- Sept. 29, '36	2.66	2.77	2.75	2.77*	2.52*	2.65	2.88	3.39*	3.47						
II	Oct. 1, '36- Oct. 6, '37	0.88	0.71	0.82	0.85	0.97	0.95	0.74	0.87	0.92	0.80	0.88	0.95	2.36	2.66	2.83
III	Oct. 7, '36- Oct. 6, '37	0.55	0.39	0.53	0.59	0.57	0.59	0.48	0.53	0.52	0.48	0.58	0.56	1.49	1.69	1.66
IV	Sept. 29, '36- Oct. 4, '37	0.32	0.18	0.19	0.44	0.32	0.35	0.24	0.33	0.34	0.33	0.35	0.68	0.76	1.06	1.36
II	July 26, '37- Oct. 6, '37	1.39	1.07	1.21	0.96	1.49	1.88	1.13	0.99	1.26	1.43	0.88	1.65	3.77	3.10	4.60
III	July 27, '37- Oct. 6, '37	1.42	0.80	1.40	1.36	1.83	1.42	1.47	1.58	1.46	1.47	1.94	1.99	4.34	5.38	5.34
IV	July 26, '37- Oct. 4, '37	0.82	0.55	0.52	1.10	0.94	0.76	0.84	0.74	0.81	1.22	0.84	0.74	2.58	2.60	2.40

* These values are merely approximate on account of the differences in defining the dimensions of Group I, upon which the calculations are based.

and not the true value, as the growth-curve of each dimension is S-shaped, and the time interval used in the calculation is not short but considerably long. Accordingly, the calculated values of the relative growth-rate correspond to those deemed as equal throughout the interval of time.

In general, the relative growth-rate is a function of time or age, decreasing with time. Recently, HAMAI (1937) has proposed the function,

$$R = r_1 t^{-m} \quad \dots \dots \dots (1)$$

where r_1 is the relative growth rate when $t=1$, and m the decline constant of relative growth-rate, for the growth of limpets. WEYMOUTH and his collaborators (1930) have proposed an equation of the form,

$$R = r_0 e^{-pt} \quad \dots \dots \dots (2)$$

for some bivalve Molluscas, where r_0 and p are the constants. For the type of growth as expressed by the equation of the monomolecular autocatalytic reaction, it stands for the following equation,

$$R = \frac{k}{1 + e^{k(t-t_1)}} \quad \dots \dots \dots (3)$$

where k is the growth constant and t_1 is the time at which the growth has reached half of the whole growth. These formulae, (1), (2) and (3), are merely several examples of this sort of formulae. At any rate, it is true that the relative growth-rate can be expressed by a function of time.

Now, as to a dimension or mass of a part of the body or of the body as a whole, these can be represented by the following formula,

$$R = \frac{1}{x} \frac{dx}{dt} = k_1 f'(t)$$

Then,

$$\log x = k_1 f(t) + c_1$$

where c_1 is the constant. Equally as to the other dimension or mass,

$$\log y = k_2 g(t) + c_2.$$

If the relation,

$$\psi(t) \cdot f(t) = g(t)$$

exists,

$$\log y = \frac{k_2}{k_1} \cdot \psi(t) \cdot (\log x - c_1) + c_2 \quad \dots \dots \dots (4)$$

On the other hand,

TABLE
Allometric

Period	Group	Const.	A	B	C	D
Period I About 15 months from hatching to the autumn in the second year.	II	α	1.059 ± 0.011	1.024 ± 0.008	0.994 ± 0.004	
		$\log b$	1.0888 ± 0.0050	1.0981 ± 0.0039	1.6799 ± 0.0016	
	III	α	1.111 ± 0.012	1.021 ± 0.009	0.968 ± 0.004	
		$\log b$	1.0964 ± 0.0050	1.1008 ± 0.0036	1.6761 ± 0.0016	
	IV	α	1.046 ± 0.007	1.033 ± 0.005	0.980 ± 0.002	
		$\log b$	1.0870 ± 0.0046	1.1024 ± 0.0035	1.6779 ± 0.0014	
	II	α	0.803 ± 0.020	0.926 ± 0.021	0.968 ± 0.035	1.101 ± 0.027
		$\log b$	1.3842 ± 0.0173	1.1933 ± 0.0185	1.2878 ± 0.0306	1.3497 ± 0.0239
Period II About 12 months. following Period I	III	α	0.713 ± 0.029	0.966 ± 0.030	1.079 ± 0.048	1.034 ± 0.047
		$\log b$	1.4722 ± 0.0308	1.1558 ± 0.0321	1.1844 ± 0.0501	1.3899 ± 0.0493
	IV	α	0.565 ± 0.045	0.612 ± 0.041	1.392 ± 0.054	1.024 ± 0.036
		$\log b$	1.7091 ± 0.0630	1.6477 ± 0.0575	2.7160 ± 0.0758	1.4185 ± 0.0496
	II	α	0.770 ± 0.036	0.875 ± 0.059	0.694 ± 0.049	1.071 ± 0.042
		$\log b$	1.4227 ± 0.0390	1.2526 ± 0.0619	1.6034 ± 0.0538	1.3843 ± 0.0456
	III	α	0.561 ± 0.035	0.986 ± 0.035	0.961 ± 0.053	1.291 ± 0.046
		$\log b$	1.6490 ± 0.0390	1.1324 ± 0.0389	1.3222 ± 0.0585	1.0895 ± 0.0512
Period III Rapidly growing period from summer to autumn.	IV	α	0.671 ± 0.091	0.639 ± 0.099	1.332 ± 0.098	1.148 ± 0.063
		$\log b$	1.5574 ± 0.1272	1.6093 ± 0.1374	2.8021 ± 0.1372	1.2411 ± 0.0878
	II	α	0.753 ± 0.036	1.145 ± 0.066	0.809 ± 0.060	0.971 ± 0.041
		$\log b$	1.4427 ± 0.0400	2.9678 ± 0.0732	1.4893 ± 0.0664	1.4787 ± 0.0457
	III	α	0.644 ± 0.035	1.079 ± 0.031	0.967 ± 0.071	1.081 ± 0.036
		$\log b$	1.5701 ± 0.0385	1.0394 ± 0.0349	1.2930 ± 0.0794	1.3159 ± 0.0395
	IV	α	0.591 ± 0.113	0.909 ± 0.143	0.837 ± 0.131	1.445 ± 0.089
		$\log b$	1.6629 ± 0.1565	1.2372 ± 0.1978	1.4765 ± 0.1813	2.8359 ± 0.1240
Period (III+IV) About 13 months containing Period III and the Period of scanty feeding, Period IV.						

32.

constants.

E	P	Q	R	X	Y	Z
0.980	1.094		1.358			
+0.007	±0.007		±0.013			
1.4335	1.2489		2.9625			
+0.0030	+0.0032		±0.0061			
0.983	1.132		1.439			
+0.007	±0.008		±0.015			
1.4340	1.2544		2.9743			
+0.0030	±0.0031		±0.0062			
0.997	1.086		1.309			
+0.004	±0.005		±0.008			
1.4362	1.2479		2.9554			
+0.0027	±0.0031		±0.0056			
1.082	0.843	0.989	1.042	0.909	1.004	1.077
±0.013	±0.028	±0.018	±0.018	±0.019	+0.025	+0.035
1.3171	1.5384	1.5538	1.3281	1.3791	1.2839	2.9147
+0.0116	±0.0241	±0.0150	±0.0158	±0.0166	+0.0217	+0.0301
1.080	0.871	0.970	0.957	0.875	1.062	1.030
±0.037	+0.017	±0.035	±0.024	±0.042	±0.019	±0.027
1.3473	1.1970	1.5578	1.4280	1.3933	1.2058	2.9890
±0.0386	+0.0176	±0.0365	±0.0253	+0.0439	±0.0520	±0.0283
1.109	0.750	1.043	1.077	1.057	1.117	2.171
+0.032	±0.031	+0.049	+0.035	+0.043	+0.052	±0.079
1.2925	1.6834	1.4102	1.2557	1.1846	1.1165	3.3382
+0.0447	±0.0179	+0.0682	±0.0184	+0.0597	+0.0729	+0.1099
1.353	0.814	0.713	0.910	1.032	0.631	1.188
±0.054	+0.054	±0.054	±0.027	±0.057	±0.059	±0.078
1.0037	1.5717	1.8726	1.4815	1.2369	1.7137	2.7872
±0.0586	+0.0590	±0.0587	±0.0294	±0.0629	±0.0650	±0.0857
0.998	1.035	1.112	1.029	1.038	1.367	1.400
±0.048	±0.041	±0.055	+0.026	±0.058	±0.094	±0.092
1.4428	1.3086	1.3923	1.3141	1.2036	2.8190	2.5565
±0.0533	±0.0487	±0.0614	+0.0289	+0.0638	+0.1045	±0.1013
0.926	1.025	0.898	0.983	1.489	1.021	0.904
±0.061	±0.061	+0.076	±0.078	±0.092	±0.085	±0.110
1.5535	1.2899	1.6484	1.3903	2.5656	1.2536	1.1511
±0.0853	+0.0848	±0.1062	±0.1084	±0.1283	±0.1189	±0.1530
1.242	0.800	0.701	0.862	0.878	0.593	1.109
±0.041	±0.048	±0.058	±0.033	±0.053	±0.083	±0.063
1.1249	1.5922	1.8719	1.5260	1.3962	1.7393	2.8782
±0.0458	±0.0535	±0.0641	±0.0367	+0.0587	±0.0914	+0.0700
1.108	0.873	0.789	0.859	0.870	0.936	0.938
±0.037	±0.017	+0.021	±0.035	±0.034	±0.028	±0.037
1.3265	1.4857	1.7317	1.5097	1.3905	1.2983	1.0375
±0.0407	±0.0184	±0.0231	±0.0390	±0.0375	±0.0310	±0.0415
0.920	0.866	0.515	0.571	1.122	0.593	0.583
±0.082	±0.076	±0.098	±0.124	±0.100	±0.146	+0.164
1.5630	1.5080	0.1634	1.9455	1.0686	1.8308	1.5971
±0.1140	±0.1058	±0.1361	±0.1722	±0.1392	±0.2018	±0.2266

$$\begin{aligned} \frac{R_2}{R_1} &= \frac{\frac{1}{y} \frac{dy}{dt}}{\frac{1}{x} \frac{dx}{dt}} = \frac{k_2 g'(t)}{k_1 f'(t)} \\ &= \frac{k_2}{k_1} \frac{\psi'(t)f(t) + \psi(t)f'(t)}{f'(t)} \dots\dots\dots (5) \end{aligned}$$

The equation (4) is the general formula of relative growth, and (5) gives the ratio between relative growth-rates of two dimensions or parts of the body, and of a part and the whole.

If, as a special case.

$$\psi(t) = a = \text{constant, viz. } \frac{g'(t)}{f(t)} = a$$

$$\log y = \frac{k_2}{k_1} a \cdot \log x + (c_2 - \frac{k_2}{k_1} a c_1)$$

$$\text{or, } \log y = \alpha \log x + b \dots\dots\dots (6)$$

$$\text{in which } \alpha = \frac{k_2}{k_1} a \quad b = c_2 - \frac{k_2}{k_1} a c_1$$

$$\text{And, } \frac{R_2}{R_1} = \frac{k_2}{k_1} a = \alpha \dots\dots\dots (7)$$

Equation (6) is the allometric equation, in which α is equal to the ratio of relative growth-rates between two dimensions or parts of the body, and between a part and the whole (7). α has been defined as the *equilibrium constant* by HUXLEY and TEISSIER (1936).

With regard to the above consideration, the allometric equation is only a special case, but never fits the growth expressing S-shaped curve. In the present case, this equation has been applied as an approximate method to the measurements with relatively small intervals of time.

The values of the equilibrium constant calculated from the allometric equation are completely coincident with the ratio between the relative growth-rates calculated from $\frac{\log x_2 - \log x_1}{t_2 - t_1}$ (Table 32). The equilibrium constant shows, essentially, the degree of growth-gradient, when it is determined between two parts. Accordingly, its changes merely suggest the changes of relative growth-rate, but do not exactly show the exact values of relative growth-rate. Therefore, the relative growth-rate itself and its ratio are both important indices for relative growth.

2. Effect of Age on Relative Growth.

The relative growth-rates during about one year of Period II in Table 31 lead us to make a comparison between those with a difference of one year in age: *i. e.* the values of Group II which are of the second year of age and those of Groups III and IV which are of the third year. The average of the values of Groups III and IV roughly gives the relative growth-rate in the third year. Considered from this point of view, it can be said that the relative growth-rate decreases by about half from the second year to the third.

In the rapidly growing period (Period III), such a great decrease does not occur, and there are some dimensions which rather increase in relative growth-rate slightly (Tables 31, 33), *viz.* the dimensions, $C+D$, P , Q , Y and the volume of the middle part of the body. In the dimensions, $C+D$ and P , the rates are probably equal, while in the dimensions, Q , Y , and the volume of the middle part an increase of 10 per cent or more occurred. $C+D$, Q , Y and the volume of the middle part of the body are a set of related dimensions in the middle part of the body. It is interesting fact that only this part increased in rate. It can be considered that the intestinal content increased and affected partially the relative rate of growth: especially considering the increase of 10 per cent on the part of the areal factors Q and Y , accordingly the volume shows an apparent increase in the relative growth-rate. And, an important factor, thus making the average rate greater, is the great rate of Group III. It is natural to consider that the activity in food-taking of Group III becomes extraordinarily high. This is also related to the size-factor, which will be later described.

TABLE 33.
Changes of relative growth-rate.

Year	L	A	B	$C+D$	E	P	Q	R	Remarks
1	7.97	9.29	8.68	8.06	7.78	9.97	12.40	12.39	Group II. July 1. (Group I)-Oct. 1, '36.
2	1.39	1.07	1.21	1.28	1.88	1.13	0.99	1.26	Group II. July 26, '37-Oct. 6, '37, Period III.
3	1.12	0.68	0.96	1.32	1.09	1.16	1.16	1.14	Group III and IV, average of R in Period III.

Supposing that Group I grew to the size of Group II from hatching, *viz.* from the beginning of July to October 1, the relative growth-rate

during this period is of the period of the most intensive growth in the 1st year (Table 33). This rate is much higher by far than that of the corresponding period in the second year. The state of decrease in the relative growth-rate from the first to the third year is hyperbolic, not fitting in the equations, (1), (2) and (3). Therefore, the allometric equation does not cover the growth of the whole life, but it is only applicable to a short time interval, as a mere approximation. This present data can be adopted by the equation (1), in which a simple correction is made. That is,

$$R = r_1 t^{-m} + R_1 \dots \dots \dots (1')$$

where R_1 means a fractional part of the relative growth-rate which has nothing to do with time or age.

The relation between the growth-gradient and age will be described in a later section.

3. Effect of Size on Relative Growth.

In the period of the initial 15 months after hatching, every dimension is correlated with size in its relative rate of growth. For it is natural, during this period, that an individual with a high relative growth-rate grows greater than one with a low rate, as the initial size of all is nearly the same. That is, Group IV has the highest rate and Group III the lowest.

That the size is partially correlated with the relative growth is what has been proved in analysing the data of each individual. It is more apparently observable in the average data. That is, the relative growth-rate of Group III, *viz.* the smaller sized group, is higher in the period of one year (Period II) following the period of 15 months after hatching (Period I) than that of Group IV, *viz.* the larger sized group. In the rapidly growing period (Period III) contained in Period II, the circumstances are completely the same.

These facts show apparently that the smaller sized group continues the relative growth-rate in order to maintain its correct size. In other words, although the smaller sized group had a smaller relative rate of growth in the initial period, it is necessarily fated to have similarly a small rate of decline in the relative growth-rate. It can be considered as probable, as a cause of this phenomenon, that the smaller sized group maintains an activity expressed in the time of youth, as far as size is connected with age: *i.e.* the smaller sized group is younger in the physiologic age. The activity in food-taking mentioned in the

previous section is also an expression of many activities which appear in a young stage of physiologic age.

4. Growth-gradient.

The decline of relative growth-rate differs in each dimension, and the growth-gradient changes with age. In the initial period after hatching the growth-centre showing the most intensive relative growth is in the head part, in every group, in the direction of the long axis. On the other hand, the gradient in the growth of depth has its centre in the posterior part. It is a fact that the change of form takes place to a great extent in the youngest stage of growth. The growth-centre in the head part during this period is a continuation of what occurs during the embryonic stage. This gradient disappears after a while. In Group II, after three months from hatching, it is inverted, the growth-centre removing from the head part to the posterior part. It can be, accordingly, said that the larval conditions maintaining the embryonic conditions does not last long, never continuing more than three months.

The difference in relative growth-rate between the growth-centre in the head and the lowest part of the tail is $7.9 \pm 1.3\%$ of that of the standard length in Group II, $12.8 \pm 1.4\%$ in Group III, and $4.9 \pm 0.8\%$ in Group IV (Table 32). In the smallest sized Group, there is the steepest gradient, and the flattest gradient exists in the greatest sized group. In the gradient of depth, its difference is $26.4 \pm 1.5\%$ in Group II, $30.7 \pm 1.7\%$ in Group III, and $22.3 \pm 0.9\%$ in Group IV: and, therefore, the difference between Groups III and IV is also significant enough.

The above facts are evidence proving the discussions regarding the size-factor mentioned in the previous sections. That is, the growth-gradient is affected by the size factor accompanying the physiologic age-factor.

In Period II, the growth-centre moved from the anterior part in the larval stage to the posterior part, *viz.* *D*-part. This centre further moved from *D*-part to *C*-part in the period of the third year (Groups III and IV). The lowest part of relative-growth exists in the anterior head part in every stage of adult fish. The slope between the growth-centre and the lowest part is $29.8 \pm 3.4\%$ in Group II, $36.6 \pm 5.6\%$ in Group III, and $42.7 \pm 7.0\%$ in Group IV, respectively, against the relative growth-rate of the standard length. These slopes are all statistically significant, but in the relative magnitude of slope, there is no significant difference mutually between the groups. However, the partial differences between the respective corresponding parts are probably significant: *i. e.* between Groups

II (2nd year) and IV (3rd year), the difference is $23.8 \pm 4.9\%$ in *A*, $31.4 \pm 4.6\%$ in *B*, and $42.4 \pm 6.4\%$ in *C*; and between Groups III and IV, there are differences of $35.5 \pm 5.1\%$ in *B* and $31.3 \pm 7.2\%$ in *C*.

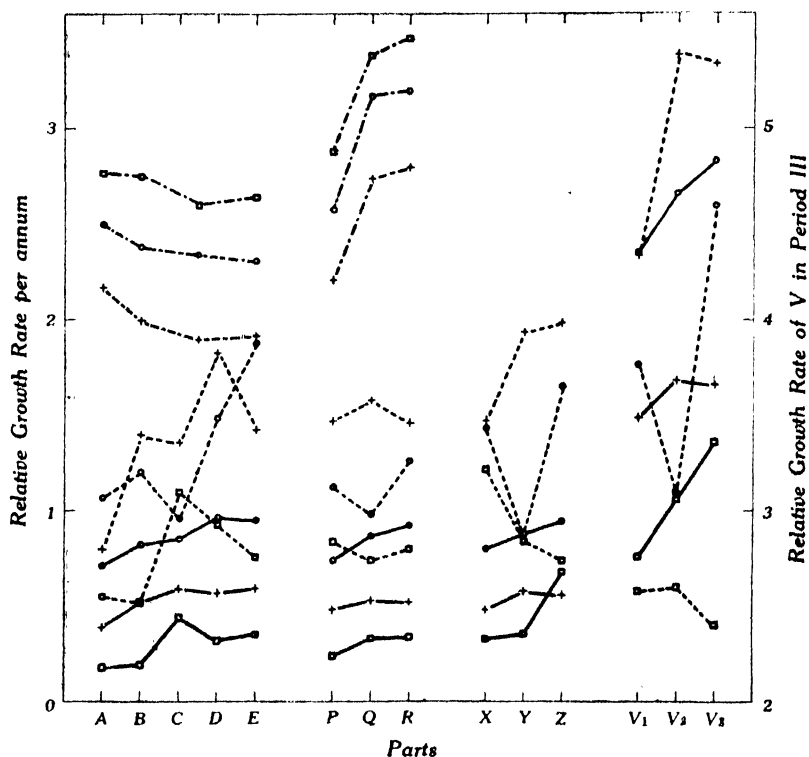


Fig. 9. Growth-gradient in various period. Dash-dot-line: Period I, full line: Period II, and broken line: Period III. \circ Group II, $+$ Group III, \square Group IV.

In depth the slope of Groups II (2nd year) and IV (3rd year) is respectively $19.3 \pm 3.3\%$ and $32.7 \pm 4.9\%$; and the centre exists in *R*, and the lowest part is *P*. In width too, the centre exists in the posterior part, *Z*, and the lowest part is *X* corresponding to *P*; and the slope is $16.8 \pm 4.0\%$ in Group II (2nd year) and $111.4 \pm 9.0\%$ in Group IV. Group III showed an insignificant gradient in depth and also in width. The difference of relative magnitude in the growth centre, *Z*, in width-gradient is strikingly great showing the values of $109.4 \pm 8.6\%$ between Groups II and IV, and of $114.1 \pm 8.3\%$ between Groups III and IV.

The growth gradients in Period II seem to be complexly influenced by many factors. The difference between Groups II and IV is subject

to the effects of size and age, and that between Groups III and IV depends mainly upon the effect of size. And, these effects have actually the same tendency: *i. e.* the relative magnitude of the relative growth-rate, as compared with that of the standard length, is higher in the younger and smaller sized group, *viz.* Group II, than in the older and greater Group IV, and equally higher in the smaller sized Group III than in the greater sized Group IV in the head part, and the relative magnitude in *C* and *Z* is entirely in an inverse relation. It seems, generally speaking, that the age and size factors, (*viz.* the physiologic age-factor in a single word), affect the growth-gradient because they decrease the relative growth-rate in the head part, and on the contrary, they increase that of the trunk in the direction of the long axis, and furthermore they increase that of the anal part in width. This conclusion coincides with that obtained from the growth-process in the larval stage. The growth-gradient in the direction of depth along the long axis remains in the same condition from the larval stage to the post-larval stage.

In the rapidly growing period, the latter part of Period II, the differences between the growth-centre and the lowest part are $58.3 \pm 6.5\%$ in Group II (2nd year), $73.0 \pm 5.8\%$ in Group III (3rd year) and $66.1 \pm 13.4\%$ in Group IV (3rd year) in the gradient of the direction of the long axis. These slopes are all significant, and the existence of the growth-gradient is, therefore, apparently recognizable. Here an interesting fact is the variation of the part in which the growth-centre exists: *i. e.* it exists in the part, *E*, in the second year, in *D* in Group III (3rd year, small size) and in *C* in Group IV (3rd year, large size). The part with the lowest relative rate of growth is probably *A* in every group. From these facts, it is probably recognizable that the physiologic age-factor conditions the location of the centre of relative growth, and that the younger the physiologic age, the more posteriorly is the centre located, in the period of intensive growth.

In depth, no significant gradient exists, but in width, there are significant slopes between *Z* and *Y*, with the difference of $55.7 \pm 9.8\%$ in Group II and between *X* and *Z* with the difference of $58.5 \pm 14.3\%$ in Group IV. These gradients in width coincide with those of the direction of length in the point of direction: *i. e.* in Group II, the centre is located in the posterior part, and in Group IV, it is in *X*, the posterior head part.

These results show a change of the growth-gradient with the season. That is, there is a tendency in which the second growth-centre appears in the head in summer season.

The change of form in the fish body can be described briefly: the head becomes smaller and the trunk and tail comparatively larger in normal growth.

5. Effect of Diet on Relative Growth.

When the diet was diminished by half, on the average no significant growth occurred. But the data of growth of individuals show, more or less, positive or negative growths. Then, in order to ascertain the change of relative growth during this period, the equilibrium constants have been examined at intervals in the time containing the period of small feeding (Period IV) and Period III. The results show some apparent changes of relative growth.

In Group II, the growth-centre in the part *E*, which was shown in Period III, remains as the primary centre, with the difference of relative magnitude in relative growth rate, $43.3 \pm 7.3\%$ between *E* and *C*, and in addition to this centre the second centre became prominent in the posterior head part *B*, with the difference of $39.2 \pm 7.5\%$ from the anterior part of the head, *A*. In Group III too, the second centre appeared in *B* with the slope of $43.5 \pm 4.7\%$ from *A*, while the primary centre removed from *D* to *E*, the slope of which is $46.4 \pm 5.1\%$ from *A*. In Group IV, the growth centre, which had been located in *C* in the previous Period III, removed to *D*, and the slope between the centre and the lowest part, *A*, is $85.4 \pm 14.4\%$. No significant change occurred in depth. The circumstances in width are similar to that in Period III: *i.e.* in Group II, between *Z* and *Y*, there is a difference of $51.6 \pm 10.4\%$, *Z* being the centre; and no significant gradient exists in Groups III and IV.

The above facts show that the lack of diet affected the growth-gradient, as the growth centre of Groups III and IV (of old age) was removed posteriorly — this is an inverse phenomenon to the case of the rapidly growing period — and the second growth-centre appeared in the posterior part of the head in the small sized group, Groups II and III. These facts indicate that, in the other parts of the body, the negative growth was slightly greater than the part *B* and the middle part of body. In the period of no diet, it has been already mentioned that negative growth occurred in most specimens. On account of the slightness of the negative growth, the change of relative growth would not clearly appear. At any rate, the form of the fish alters for the head becomes relatively large and the trunk and tail relatively small in the starving conditions. The primary centre of growth in the middle part of the body shown in the normal

condition has the tendency to fall off to the negative centre, *viz.* the lowest part of relative growth-rate, in the starving conditions.

VI. VARIATION OF SIZE.

1. General.

A group in which all individuals are of the same age is, in many cases, subject to the law of variation expressing the normal distribution in many characteristics. When two kinds of dimensions of a body are considered, the degree of correlation of this pair of two variables is usually expressed by the correlation-coefficient. This index does not give the direction of variation. In the simplest complete correlation, all points corresponding to the measurements are arranged strictly on a straight line. This straight line is expressed by the equation,

$$y = \frac{\sigma_y}{\sigma_x} x + (y_0 - \frac{\sigma_y}{\sigma_x} x_0) \dots \dots \dots (8)$$

where σ_x and σ_y are respectively the standard deviation of x and y , and, x_0 and y_0 are respectively the mean of x and y . In other words, this equation (8) is that in coincidence of two equations of regression-line,

$$x - x_0 = r \frac{\sigma_x}{\sigma_y} (y - y_0)$$

and
$$y - y_0 = r \frac{\sigma_y}{\sigma_x} (x - x_0)$$

in the case of $r=1$. When the coefficient of correlation r is approximate to 1 or favourably high value, it is probably reasonable that the equation,

$$y = px + q \dots \dots \dots (9)$$

which is the same form as equation (8), is applied to the pairs of measurements. HAMAI (1938) has successfully applied this equation to the data of a Mollusca, and has studied the direction of variation changing with age. The constant p is, then, approximately equal to $\frac{\sigma_y}{\sigma_x}$, *i. e.*

$$p = \frac{\sigma_y}{\sigma_x} \dots \dots \dots (10)$$

Therefore, p is a ratio between the magnitude of variation of a dimension and that of another dimension being in close correlation of the primary order with the former. Consequently, p is necessarily correlated with the coefficient of variation, which is defined as $\frac{\sigma}{M} \times 100$. The coefficient of variation generally increases with advance in age. In fact, it increased

TABLE

Direction-coefficient of variation and the other

Group	Date	No.	Const.	A	B	C	D	E
I	July 1, 1936	30	p	0.097 ± 0.035	0.091 ± 0.027	0.535 ± 0.042		0.277 ± 0.045
			q	0.017 ± 0.025	0.024 ± 0.020	-0.040 ± 0.031		-0.002 ± 0.033
	Oct. 1, 1936	19	p	0.128 ± 0.013	0.144 ± 0.010	0.226 ± 0.021	0.271 ± 0.026	0.236 ± 0.011
			q	0.25 ± 0.08	-0.04 ± 0.06	-0.25 ± 0.13	-0.02 ± 0.16	0.02 ± 0.06
	July 26, 1937	6	p	0.125 ± 0.005	0.135 ± 0.009	0.173 ± 0.015	0.300 ± 0.008	0.267 ± 0.012
			q	0.31 ± 0.06	-0.03 ± 0.10	0.23 ± 0.17	-0.15 ± 0.09	0.36 ± 0.14
II	Oct. 6, 1937	6	p	0.130 ± 0.005	0.144 ± 0.005	0.139 ± 0.007	0.292 ± 0.015	0.295 ± 0.013
			q	0.19 ± 0.09	-0.22 ± 0.08	0.57 ± 0.11	0.00 ± 0.24	-0.54 ± 0.21
	Aug. 18, 1938	6	p	0.130 ± 0.009	0.156 ± 0.012	0.146 ± 0.013	0.289 ± 0.009	0.278 ± 0.007
			q	0.20 ± 0.16	-0.30 ± 0.20	0.61 ± 0.22	-0.23 ± 0.15	-0.27 ± 0.13
	Jan. 5, 1939	6	p	0.126 ± 0.005	0.172 ± 0.005	0.105 ± 0.018	0.311 ± 0.013	0.287 ± 0.013
			q	0.35 ± 0.08	-0.56 ± 0.09	1.13 ± 0.32	-0.69 ± 0.23	0.27 ± 0.22
	Oct. 7, 1936	20	p	0.129 ± 0.007	0.122 ± 0.007	0.201 ± 0.013	0.282 ± 0.015	0.266 ± 0.010
			q	0.25 ± 0.05	0.06 ± 0.05	-0.12 ± 0.10	-0.19 ± 0.11	0.00 ± 0.07
	July 27, 1937	15	p	0.085 ± 0.007	0.133 ± 0.004	0.191 ± 0.009	0.312 ± 0.015	0.279 ± 0.009
			q	0.73 ± 0.07	-0.05 ± 0.04	-0.06 ± 0.10	-0.61 ± 0.15	-0.02 ± 0.09
III	Oct. 6, 1937	11	p	0.102 ± 0.004	0.118 ± 0.004	0.170 ± 0.010	0.327 ± 0.013	0.283 ± 0.011
			q	0.49 ± 0.05	0.20 ± 0.06	0.22 ± 0.13	-0.80 ± 0.18	-0.11 ± 0.15
	Aug. 20, 1938	11	p	0.100 ± 0.005	0.132 ± 0.006	0.179 ± 0.013	0.274 ± 0.009	0.315 ± 0.010
			q	0.61 ± 0.07	0.07 ± 0.08	-0.13 ± 0.17	-0.15 ± 0.13	-0.42 ± 0.14
	Jan. 6, 1939	11	p	0.090 ± 0.009	0.129 ± 0.007	0.168 ± 0.013	0.317 ± 0.017	0.295 ± 0.012
			q	0.81 ± 0.13	0.17 ± 0.11	-0.02 ± 0.20	-0.84 ± 0.25	-0.11 ± 0.17
	Sept. 29, 1936	10	p	0.069 ± 0.021	0.151 ± 0.015	0.238 ± 0.042	0.234 ± 0.038	0.308 ± 0.035
			q	1.39 ± 0.40	-0.22 ± 0.29	-1.39 ± 0.82	0.94 ± 0.74	-0.73 ± 0.69
	July 26, 1937	10	p	0.087 ± 0.023	0.121 ± 0.035	0.152 ± 0.040	0.402 ± 0.031	0.237 ± 0.048
			q	0.95 ± 0.54	0.23 ± 0.81	0.64 ± 0.93	-2.87 ± 0.72	1.08 ± 1.11
IV	Oct. 4, 1937	9	p	0.086 ± 0.032	0.066 ± 0.028	0.357 ± 0.040	0.276 ± 0.049	0.215 ± 0.039
			q	0.97 ± 0.85	1.56 ± 0.76	-4.52 ± 1.09	0.22 ± 1.33	1.77 ± 1.06
	Aug. 17, 1938	7	p	0.152 ± 0.067	0.094 ± 0.076	0.215 ± 0.111	0.342 ± 0.140	0.201 ± 0.090
			q	-0.84 ± 1.75	0.87 ± 2.00	-1.04 ± 2.92	-1.25 ± 3.67	2.15 ± 2.35
	Jan. 5, 1939	7	p	0.076 ± 0.046	0.148 ± 0.068	0.365 ± 0.085	0.182 ± 0.116	0.229 ± 0.049
			q	-1.18 ± 1.21	-0.41 ± 1.79	-5.00 ± 2.23	2.65 ± 3.04	1.59 ± 1.29
	Oct. 6, 1936	25	p	0.101 ± 0.017	0.202 ± 0.023	0.078 ± 0.039	0.266 ± 0.036	0.354 ± 0.023
			q	0.72 ± 0.28	-0.94 ± 0.39	1.54 ± 0.64	0.04 ± 0.59	-1.37 ± 0.39
	Aug. 19, 1938	17	p	0.153 ± 0.004	0.130 ± 0.003	0.205 ± 0.013	0.241 ± 0.012	0.271 ± 0.007
			q	0.12 ± 0.02	0.03 ± 0.02	-0.15 ± 0.08	0.00 ± 0.08	-0.01 ± 0.05
VI	Jan. 7, 1939	9	p	0.162 ± 0.010	0.152 ± 0.007	0.190 ± 0.013	0.244 ± 0.018	0.312 ± 0.015
			q	0.06 ± 0.07	-0.12 ± 0.05	-0.08 ± 0.10	-0.15 ± 0.14	-0.15 ± 0.11

34.

constant in the relative variation equation.

P	Q	R	X	Y	Z
0.153 ± 0.032 0.014 ± 0.023	0.132 ± 0.046 -0.005 ± 0.033	0.041 ± 0.029 0.029 ± 0.021			
0.339 ± 0.027 -0.45 ± 0.16	0.451 ± 0.013 -0.58 ± 0.08	0.274 ± 0.013 -0.26 ± 0.08	0.243 ± 0.010 -0.23 ± 0.06	0.262 ± 0.013 -0.40 ± 0.07	0.101 ± 0.011 -0.04 ± 0.06
0.206 ± 0.020 0.39 ± 0.23	0.430 ± 0.012 -0.56 ± 0.15	0.290 ± 0.017 -0.46 ± 0.20	0.225 ± 0.022 0.39 ± 0.25	0.253 ± 0.017 -0.38 ± 0.20	0.107 ± 0.009 -0.11 ± 0.11
0.208 ± 0.006 0.28 ± 0.10	0.371 ± 0.005 -0.33 ± 0.07	0.260 ± 0.004 -0.31 ± 0.07	0.214 ± 0.006 -0.36 ± 0.10	0.212 ± 0.009 -0.24 ± 0.14	0.082 ± 0.006 0.27 ± 0.09
0.222 ± 0.011 0.13 ± 0.20	0.319 ± 0.006 0.05 ± 0.10	0.232 ± 0.004 -0.06 ± 0.07	0.212 ± 0.012 -0.46 ± 0.21	0.192 ± 0.009 -0.21 ± 0.15	0.108 ± 0.006 -0.07 ± 0.09
0.219 ± 0.009 0.08 ± 0.16	0.293 ± 0.003 0.17 ± 0.06	0.235 ± 0.004 -0.25 ± 0.07	0.222 ± 0.008 -0.59 ± 0.13	0.200 ± 0.011 -0.52 ± 0.19	0.109 ± 0.005 -0.27 ± 0.08
0.225 ± 0.003 0.13 ± 0.03	0.325 ± 0.016 0.08 ± 0.12	0.254 ± 0.010 -0.10 ± 0.08	0.174 ± 0.009 0.12 ± 0.06	0.185 ± 0.009 -0.02 ± 0.07	0.122 ± 0.006 -0.16 ± 0.05
0.187 ± 0.004 0.35 ± 0.05	0.336 ± 0.012 -0.12 ± 0.13	0.255 ± 0.006 0.22 ± 0.06	0.148 ± 0.005 0.31 ± 0.05	0.189 ± 0.008 -0.15 ± 0.08	0.102 ± 0.003 -0.08 ± 0.04
0.191 ± 0.005 0.45 ± 0.07	0.284 ± 0.011 0.60 ± 0.15	0.241 ± 0.006 -0.06 ± 0.08	0.167 ± 0.009 0.11 ± 0.12	0.184 ± 0.010 0.05 ± 0.14	0.108 ± 0.003 -0.05 ± 0.04
0.184 ± 0.005 0.49 ± 0.06	0.270 ± 0.009 0.45 ± 0.12	0.207 ± 0.005 0.12 ± 0.07	0.145 ± 0.004 0.44 ± 0.05	0.158 ± 0.005 0.11 ± 0.07	0.092 ± 0.003 -0.01 ± 0.05
0.189 ± 0.005 0.35 ± 0.08	0.254 ± 0.015 0.39 ± 0.22	0.223 ± 0.007 -0.21 ± 0.11	0.165 ± 0.008 0.15 ± 0.12	0.157 ± 0.013 -0.08 ± 0.20	0.115 ± 0.010 -0.48 ± 0.15
0.128 ± 0.027 1.98 ± 0.54	0.261 ± 0.057 1.03 ± 1.13	0.219 ± 0.027 0.15 ± 0.53	0.224 ± 0.037 -0.84 ± 0.73	0.246 ± 0.052 1.20 ± 1.03	0.061 ± 0.014 0.20 ± 0.27
0.146 ± 0.026 1.49 ± 0.60	0.103 ± 0.068 5.07 ± 1.56	0.223 ± 0.047 0.24 ± 1.08	0.138 ± 0.029 0.76 ± 0.66	0.183 ± 0.048 0.20 ± 1.11	0.009 ± 0.028 -2.63 ± 0.66
0.231 ± 0.034 -0.53 ± 0.91	0.337 ± 0.072 -0.52 ± 1.95	0.266 ± 0.040 -0.90 ± 1.09	0.139 ± 0.030 1.23 ± 0.80	0.257 ± 0.026 -1.75 ± 0.72	0.111 ± 0.017 -0.21 ± 0.46
0.192 ± 0.076 0.46 ± 1.99	0.365 ± 0.082 -1.86 ± 2.16	0.375 ± 0.069 -4.25 ± 1.80	0.188 ± 0.078 -0.30 ± 2.04	0.251 ± 0.079 -1.96 ± 2.06	0.054 ± 0.065 1.31 ± 1.69
0.146 ± 0.083 1.84 ± 2.17	0.351 ± 0.084 -1.71 ± 2.19	0.303 ± 0.071 -2.25 ± 1.86	0.259 ± 0.077 -2.10 ± 2.01	0.322 ± 0.050 -3.94 ± 1.32	0.050 ± 0.016 1.17 ± 0.43
0.243 ± 0.024 -0.07 ± 0.40	0.270 ± 0.034 0.62 ± 0.57	0.198 ± 0.029 0.41 ± 0.49	0.156 ± 0.019 0.34 ± 0.32	0.169 ± 0.027 -0.14 ± 0.45	0.066 ± 0.019 0.40 ± 0.32
0.221 ± 0.007 0.10 ± 0.04	0.332 ± 0.010 -0.09 ± 0.06	0.243 ± 0.008 -0.17 ± 0.05	0.177 ± 0.004 0.09 ± 0.02	0.180 ± 0.009 -0.04 ± 0.06	0.099 ± 0.005 -0.05 ± 0.03
0.201 ± 0.006 0.18 ± 0.05	0.254 ± 0.012 0.24 ± 0.09	0.189 ± 0.009 0.10 ± 0.07	0.161 ± 0.004 0.15 ± 0.03	0.141 ± 0.015 0.10 ± 0.11	0.072 ± 0.008 -0.03 ± 0.06

from 4.5 ± 0.4 (30 specimens of Group I) to 82.1 ± 6.6 (49 specimens in the mixture of Groups II, III and IV) in the standard length after about 15 months from hatching. p is, therefore, an index comparing the state of the extending variation with ageing between two dimensions or parts. Here, p can be defined as the *coefficient of direction of variation*. In the present case of the fish, the equation (9) has been used for the study of the direction of variation between two comparable dimensions (Table 34). As the standard the standard length was taken. The direction-coefficient of variation is practically more convenient than the pair of regression coefficient, $r \frac{\sigma_r}{\sigma_y}$ and $r \frac{\sigma_y}{\sigma_r}$, for p is a single index, which gives the direction of variation, to compare the direction of one dimension with others, and to compare the direction of variation of one dimension at a certain time with that at another time.

Of the present cases, several, especially of the largest Group IV, showed insignificant values of p , because of the small number of observations and of the comparatively great deviations, but in many cases the values of p were significant (Table 34).

2. Age, Size and Direction of Variation.

In the dimensions, C , P , Q , R and Y in Group II; in P , Q , R and Z in Group III; and in Q and R in Group VI, decreases of the values of p were significantly found with advance in age. In the other dimensions in every group no significant difference was observed. It is suggested that the direction of variation in a dimension against the standard length is correlated with age, and decreases its values with advance in age. Then, comparing the values of p between groups at a similar phase of season (Table 35), a definite tendency of decreasing values is found in the dimensions A , B , P , Q , X and Y between Groups II and III, and in P between II and IV, in all the values of p having a significant difference, with an exception in the case of C between Groups II and IV. Group II is younger than Groups III and IV. Accordingly, the definite tendency of decreasing p is generally parallel with the advance in age. There can be seen that the effect of age is directed on decreasing the direction-coefficient of variation.

If the values of P , Q and R in Groups II, III and VI are plotted against the age, those of Groups II and III are on the same trend, and the values of Group VI are on another trend (Fig. 10). Synthesizing these trends, *viz.* the decreasing values of p with ageing, and the dif-

TABLE 35.
Differences of direction-coefficients of variation.

	Dimension	Group (time (phase))	Differences
		young aged group — old aged group	
Inter-group differences at the same phase of season	A	II (July 26, '37) — III (July 27, '37)	.040 ± .009
	A	II (Oct. 6, '37) — III (Oct. 6, '37)	.028 ± .006
	B	II (Jan. 5, '39) — III (Jan. 6, '39)	.043 ± .009
	C	II (Oct. 6, '37) — IV (Oct. 4, '37)	— .218 ± .041
	P	II (Oct. 1, '36) — III (Oct. 7, '36)	.114 ± .027
	P	II (Oct. 1, '36) — IV (Sept. 29, '36)	.211 ± .038
	Q	II (Oct. 1, '36) — III (Oct. 7, '36)	.126 ± .021
	Q	II (July 26, '37) — III (July 27, '37)	.094 ± .017
	Q	II (Oct. 6, '37) — III (Oct. 6, '37)	.087 ± .012
	Q	II (Aug. 18, '38) — III (Aug. 20, '38)	.049 ± .011
	X	II (Oct. 1, '36) — III (Oct. 7, '36)	.069 ± .013
	X	II (Oct. 6, '37) — III (Oct. 6, '37)	.047 ± .011
	X	II (Aug. 18, '38) — III (Aug. 20, '38)	.067 ± .013
Inter-group differences at the same age	X	II (Jan. 5, '39) — III (Jan. 6, '39)	.057 ± .012
	Y	II (Oct. 1, '36) — III (Oct. 7, '36)	.126 ± .021
		small sized group — large sized group	
	C	III (Oct. 6, '37) — IV (Oct. 4, '37)	— .167 ± .041
	P	II (Oct. 1, '36) — VI (Aug. 19, '38)	.118 ± .028
	Q	II (Oct. 1, '36) — VI (Aug. 19, '38)	.119 ± .016
	X	II (Oct. 1, '36) — VI (Aug. 19, '38)	.066 ± .011
	Y	II (Oct. 1, '36) — VI (Aug. 19, '38)	.082 ± .016
	Z	II (Oct. 6, '37) — III (Oct. 7, '36)	.040 ± .008

ference between the young and old groups, it is decidedly concluded that the advance in age diminishes the values of p in many dimensions, especially in P , Q and R .

Secondly, comparing the values of p between groups, at a similar phase in the same age (Table 35), differences were significantly observed in some dimensions. This fact shows an apparent effect of size on the values of p . This effect is one of decreasing the values, upon which the increasing of size gives. The effect of size explains the different trend of decrease between Groups II and VI above mentioned: *i.e.* although Groups II and VI are of the same age, Group II is smaller than Group VI, and therefore, the size-effect plays the rôle. But there is a case of

inverse result in *C* between Groups III and IV. The values of Group IV were, however, generally variable and the probable errors were relatively great. The size-effect is reasonably considered as the effect of physiologic age as has been already discussed in the previous chapter.

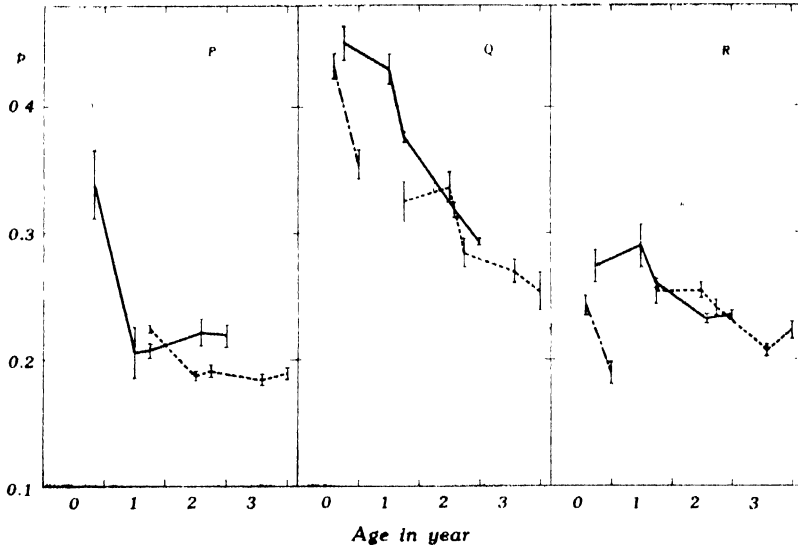


Fig. 10. Decreasing tendency in the value of *p*. The full line indicates Group II, the broken line Group III, and the dash-dot Group VI.

3. Growth and Variation in Size.

The variation in size occurs from the difference of growth. There is, however, a variation already in the stage of the egg-cell. This variation in the egg-stage can be reasonably considered as originating from the growth-difference of the oocyte in the course of oogenesis. But, it seems that the variation, in the stages of such an early development, is very small. It is assumed from the fact that the variation of the larval stage is small compared with the adult stage, as mentioned above. Thus, the coefficient of direction in variation, expressing the state of variation of two dimensions or parts, must be closely related with growth. When the direction-coefficient of variation is seen as a series along the axis, there is found a definite gradient (Fig. 11, 12 and 13). On this gradient, the smallest values are on the head parts, *A* and *B*, and the greatest exist on the posterior parts, *D* and *E*, in the dimensions parallel to the long axis: in depth the middle part *Q* is the greatest, and in width the head and the middle part of the trunk are equally high, and the posterior

the lowest. The state of this gradient does not change except in some small deviation throughout the period of growth. The gradient of Group IV was unknown on account of its insignificant values of p .

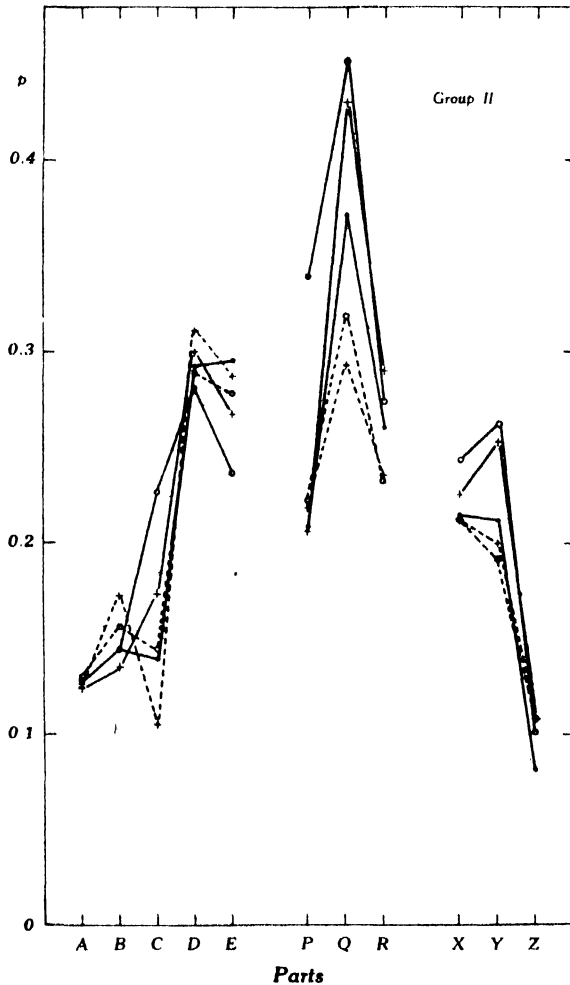


Fig. 11. Gradient of the value of p along the length-axis. Data at various times are together plotted.

Such a definite gradient mentioned above is almost parallel with the gradient of the absolute growth-rate (Fig. 4), and different from the gradient of relative growth-rate (Fig. 9).

A pair of size being x_1 and y_1 , at the beginning of growth, and the

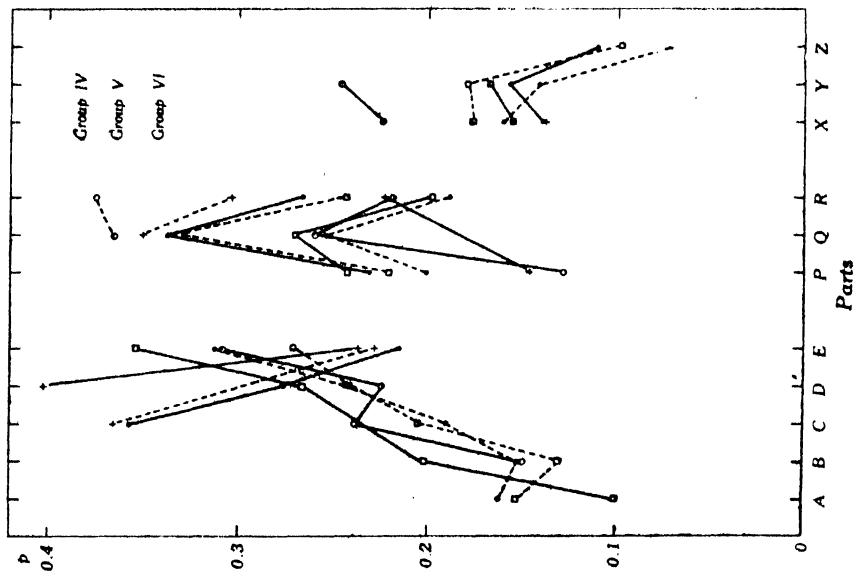


Fig. 12.

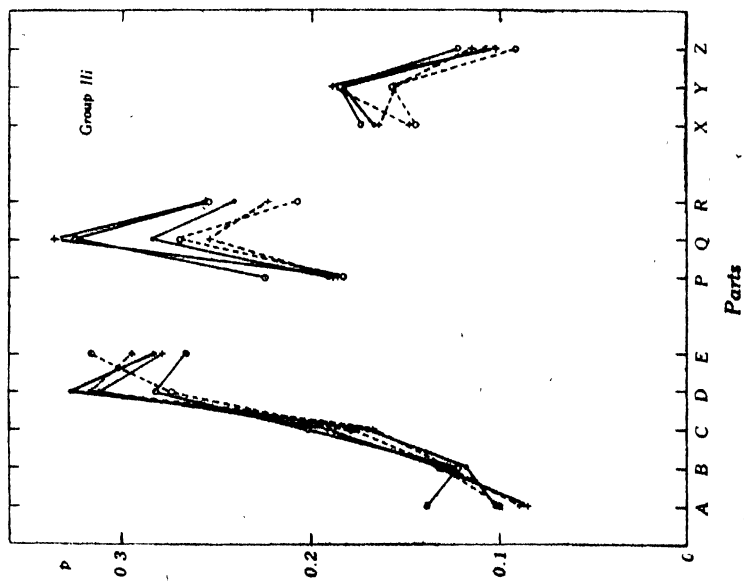


Fig. 13.

Fig. 12 and 13. Gradient of the value of p along the length-axis. Data at various times are together plotted.

pair of size x and y being obtained at an instant as the result of growth,

$$x = x_1 + x_i$$

$$y = y_1 + y_i$$

where x_i and y_i are the increases during the period from the beginning to the instant. From equation (9),

$$y_1 + y_i = p(x_1 + x_i) + q$$

$$\frac{y_i}{x_i} = \frac{px_1 + q - y_1}{x_i} + p$$

When the values of p and q are finite and $px_1 + q - y_1 \neq 0$,

$$\lim_{x_i \rightarrow \infty} \frac{y_i}{x_i} = p$$

and also $\frac{y_i}{x_i}$ approaches $\frac{dy}{dx}$, when $x_i \rightarrow \infty$: i. e. when the increment of x enlarged infinitely, the ratio of the absolute growth rates, $\frac{dy}{dt} / \frac{dx}{dt}$, approaches p . But the growth, in this case, is assumed as tracing a straight line beginning from the initial point (x_1, y_1) : i. e. it is measured assuming that $\frac{dy}{dt} / \frac{dx}{dt} = \text{constant}$. Then, because the initial size is very small, the ratio of the absolute growth-rates, measured from the initial point, is reasonably deemed as approximately equal to the value of p , when a considerably large size is attained.

In consequence of the above theoretical consideration, it is very natural that the gradient of the direction-coefficient of variation is nearly similar to the gradient of absolute growth-rate. The direction-coefficient of variation, here, extends its meaning, containing a significance as the relative ratio of the absolute growth-rate at a considerably large size. In other words, the variation as a result of growth can be strictly explained by the direction-coefficient of variation from the point of view of growth, and furthermore the state of variation can be briefly and also exactly described by this coefficient.

VII. DISCUSSIONS.

1. Natural Growth.

Under natural conditions the growth of fishes is made up of annual cycles of growth. It is already described in Chapter III that some fishes, such as plaice, haddock, as well as the carp in the present case, show

this sort of cyclic growth. DANNEVIG (1933) has also observed such a growth in the cod, *Gadus callarias*. This annual cycle is largely correlated with the temperature of the medium. In the case of plaice, haddock, and carp, low temperature retards the growth. On the other hand, in the case of cod, low temperature does not retard the growth, but, an intensive growth is observed in the period of low temperature. In this case high temperature retards the growth. These mutually contradictory facts suggest the existence of an optimum temperature acting on the species with specific influences.

When the annual cycle of growth is neglected, or when only the same phase of each cycle is taken into consideration, or when the growths during a year, two years, three years and so on, are plotted against age, the curve of growth is drawn as a sort of parabolic curve in the length-growth, and as a sigmoid curve in the weight-growth. EBINA (1936) found this fact in *Pagrosomus major*. In his case, the age was determined by the number of the annual rings in the scale, a ring being equivalent to a year. On the other hand, PAULSEN (1933) has observed the S-shaped growth-curve in the dab in the length too. The external environments and the internal specific nature may vary the growth. According to MATUI (1938), the length-growth of the carp, in the condition of rearing, obeys the formula —

$$L = \frac{t-1}{0.0176t+0.4905} + 1.45$$

and the weight grows obeying a simple type of the logistic curve,

$$W = \frac{1473}{1 + e^{3.3112 - 0.0998t}}$$

in which formulae the age t is measured by the unit of months. It was also found that the other fishes, e. g. *Carassius auratus* and its variety obeyed the same forms of the formulae. According to MATUI's formula regarding the length, the size at the ages of 12 and 24 months is obtained as follows:—

	MATUI's specimen	The specimen in this paper	
		average	larger specimen
12 months	17.1	10.9	
24 months	27.1	18.6	23.1

MATUI's observation shows much greater size than in the present writer's case. KUROKI (1939) found, under natural conditions, the size of the carp to be similar to the larger sized group in the writer's case in Lake

Kizaki, Aoki and Nakatuna, *i.e.* in the specimens of the second and third years : —

	Lake Kizaki				Lake Aoki		Lake Nakatuna
	April	May	June	July	June	July	July
2nd year	12.5	12.8	15.7	16.1	13.2	15.0	13.0
3rd year	21.1	19.4	—	31.2			

In this case the age was determined by the number of annual rings. In the above tables, the specimen of the 3rd year caught in Lake Kizaki in July is larger than MATUI's specimens, and remarkably larger than the present writer's. This individual may be a giant-like variation. In the present specimens the larger sized group is, consequently, of the ordinary size, considerably well developed. From the above fact it is clearly found that the variation of size is very great. WIELENBACH (1938) suggested that the growth of the carp is subject to the hereditary factor limiting its growth-rate and size.

The effect of crowding upon growth has been studied by several workers, and they have obtained the results that, even when the living space of equal size is given to each individual, much crowding harmfully affects the growth-rate. HOFFBAUER observed the effect of this on the growth of the carp. BILSKI has proposed the formula expressing the relation between the size of animals and their numbers, *i.e.*

$$y = k \left(\frac{\sqrt{x}}{x-1} \right)$$

where y represents size, x stands for the number of animals in a given space, and k is a constant. According to BILSKI, values calculated from this formula fit fairly well with his observations of tadpoles and with HOFFBAUER's observations of the carp (*vide* ALLEE, 1931). Accordingly, care must be given to the size of the medium in the rearing experiments. In the present case of experiments, the larger specimens, few in number, were taken in the pond of greater volume (Table 1). To eliminate the effect of the accumulation of toxic substances, and to equalize the conditions of the medium, *e.g.* the oxygen tension, carbon-dioxide tension, etc., running water was always used, so that the effect of over-crowding would probably be eliminated. Even though the effect was active, it would be negligible. At any rate, growth took place normally and the differences of growth-feature appeared between the various groups.

2. Relative Growth.

The relative growth of fish has, up to the present time, been described by the formula of the straight line,

$$y = mx + c \quad \dots\dots\dots (11)$$

where y is a dimension of a part, x is, in many cases, the standard length or total length, m the rate of increase in y per increase of x , and c a constant. CROZIER and HECHT (1913) have reported about *Cynoscion regalis* that the standard length, body length, tail length, head length, depth, and width are expressed by a straight line against the total length, and accordingly, by means of comparison of the tangents, m , in (11) between the parts, that the body has by far the greatest rate of growth, while the width has the least, and that the head and tail have approximately the same rates of growth, and that the depth and width also grow at about the same rate. The rate given above is not the ratio of relative growth-rate, but that of absolute growth-rate. HECHT (1916) has also studied the growth of several fishes by means of the same method.

In the case in which the relation between a part and the whole body was expressed by a straight line (11), it can clearly be concluded that when the constant c has a considerable magnitude, the allometry is shown, and that, when c is zero, the growth is isometric. According to HECHT (1916), the isometric growths appear for a long time of growth in the cases of *Brevoortia tyrannus*, *Anchovia brownii*, *Anchovia mitchilli*, *Peprilus alepidotus*, *Leiostomus xanthurus*, and *Orthopristis chrysopterus*, as the values of c are practically zero in all cases. Therefore, the change of the form in the fish body does not occur in the course of growth. HAMMETT and HAMMETT (1939) have studied the relative growth of *Lepisosteus platyrhincus*, and stated that the form of *Lepisosteus platyrhincus* is not constant and the rates of growth of all parts are not identical. In this case, having used the equation of the straight line (11) the constant c was not zero but was of a considerable size. Accordingly, the growth of any part is allometric against the standard length. In *Carassius auratus* and its variety, the changes of the ratios between the body-length and the tail-length and between the body-length and the width have been reported by KATO (1932). This also shows the allometric growth of parts. The records of some fishes measured by AIKAWA and KATÔ (1938) give many allometric results in the head and the other parts against the standard length by means of the present writer's calculations (Table 36).

result, which will be later discussed. MOTTLEY (1936) has studied, in detail, the relative growth of various parts of the body in *Salmo kamloops*, also by means of the allometric equation. In the case of Scorpaenoid fish, *Sebastes inermis*, the fitness of the allometric formula is also suggested for a long period of growth, observing from the figures given by MATSUBARA (1935), in the head-length, maxillary-length, diameter of orbit, depth of body, length of the longest dorsal spine, length of the second anal spine, and the length of pectoral fin in relation to the body-length. It is, thus, certainly found that the phenomena of allometry are widely distributed in all parts of the fish-body.

In general, the degree of allometry is different according to the parts, and it changes with age. The growth-gradient and its change according to age is thus proved. In the young fish, there exists the gradient directed from the head to the tail. In the embryonic development, the head grows at first intensively. This intensive growth of the head affects the growth in the period following and would remain till the stage of young fish. But, after attainment of a certain size, or after the larval stage, the relative growth-rate suddenly falls off, and the lowest relative growth of the head part appears during the adult stage. The positive allometry of the head in the young stage is also observed in the case of *Thynnus orientalis* and *Seriola aureovittata* (Table 36). The negative allometry in the adult stage is also seen in the case of *Mustelus canis* (KEARNEY, 1914), *Germo germo* and *Seriola quinqueradiata*. In *Salmo kamloops*, the allometry of the head is negative in the young stage but becomes positive after a certain size is attained (MOTTLEY, 1936). In this case the conditions are entirely inverse. According to KEARNEY (1914), the brain and the eyeball follow the negative allometry of the head. MOTTLEY observed the negative growth of the diameter of the eye, in *Salmo kamloops*. Of the head, the anterior part is higher in the relative growth than in the posterior. This comparison was clearly made in the data of the writer's case in the young stage. NEEDHAM (1935) has reported the high relative growth of the jaw in *Lepidosteus osseus*, *Hemirhamphus unifasciatus*, *Belone vulgaris* and in *Hemirhamphus far* at the young stage. He has suggested the larval adaptation for the behaviour of food-capture. Thus high positive allometry may apparently be governed by the growth-centre in the head of the young. This high relative growth in the head may be a general feature among fishes with a few exceptional cases. In the case of *Salmo kamloops*, the snout shows at first an isometry and later comes to show a negative allometry.

The growth-centre moves with growth from the head to a part of the trunk, in the carp. The existence of the centre in the trunk was also observed in the case of *Seriola quinqueradiata* (Table 36). Here, an interesting fact was found regarding the locality of the centre of growth, *i.e.* during the period of the most intensive growth the centre lies in the tail in the fish of the second year, in the posterior part of the trunk in the smaller fish of the third year, and in the anterior part of the trunk in the greater fish of the third year. This fact shows that the greater the relative growth-rate, the more posterior is the growth centre. This is apparently explained by the facts found by KEARNEY (1914) in the dogfish, *i.e.* while the skeleton, viscera, spinal cord, heart, pancreas, liver, spleen, rectal gland, kidney, testes, ovary, stomach and intestine, that is almost all the organs, represent the negative allometry, after the attainment of a certain size shortly after birth; on the contrary the musculature only, comprising a great part of the fish-body, shows a positive allometry: the tail-part contains relatively a great part of the musculature, and the trunk ranks next to the tail in percentages of musculature. That is to say, the parts holding a high percentage of the musculature have necessarily the most intensive growth-rate on account of the massive growth of the musculature. In the average data during a year, a relatively high growth-rate was observed in the tail. In this feature of growth, the age factor, especially the physiologic age factor, also influences this phenomenon. Group III was similar in size to Group II which is younger in age, in the rapid growth-period, and the relative growth-rate is almost the same, as a whole. Now, Group III had its growth-centre in the posterior part of the trunk, while Group II had it in the tail. Here the physiologic age factor affected the intensive growth of musculature in the direction falling off.

In the period of scanty diet, the decrease of the content in the stomach and intestine, and the slowing down of the growth-rate of the musculature affected the growth-gradient: the second centre appeared in the posterior part of the head, which represents relatively little change, as this part mainly consists of the skeleton, which shows a slight negative allometry according to KEARNEY. HAMMETT and HAMMETT (1939) has shown in *Lepisosteus platyrhincus*, a gradient in the order of decreasing growth-rate, *viz.* body, tail, head, caudal fin. This gradient is generally coincident with the case of the carp.

In depth, the distinct growth-gradient could not be observed on account of the extensive variation of the measurements. On the other hand, the

width showed a growth-gradient which in the posterior part is high. But the group of the greatest size showed the gradient high in the anterior part. The explanation of this gradient of width along the length axis is entirely the same as mentioned above. Regarding the volumes of every part the same consideration may be given.

3. Thickness of the Body.

The relation of weight to length has been studied by many workers, up to the present time. HECHT (1913) observed the relation that the weight is proportional to the cube of the length, *viz.*

$$W = aL^3 \quad \dots\dots\dots (12)$$

CROZIER and HECHT (1913) have also observed this relation in *Cynoscion regalis*, and HECHT (1916) and KATO (1932) have further confirmed it in several other fishes. HECHT suggested that there is a constancy of form within the species studied by him, which is adhered to throughout the life of the individual.

Furthermore, CROZIER and HECHT (1913) and HECHT (1916) have established the relation that

$$\text{Weight} = K (\text{length}) (\text{width}) (\text{depth}) \quad \dots\dots\dots (13)$$

And HECHT (1916) has derived the relation

$$K = \frac{a}{c_1 c_2} \quad \dots\dots\dots (14)$$

from (12) and (13), where $c_1 = (\text{depth})/(\text{length})$, $c_2 = (\text{width})/(\text{length})$ and K a constant.

On the other hand, it is found that the weight is not proportional to the cube of the length, but to the n th power of the length. The work of KEYS (1928) indicates that in herrings, sardines, and *Fundulus* the weight increases faster than the cube of the length, which implies form-change contrary to HECHT's results, and somewhat similar results have been obtained by HICKLING (1930) with regard to the dogfish, *Acanthias vulgaris* (*vide* HUXLEY, 1932). MATUI's (1938) results in *Cyprinus carpio*, and in *Carassius auratus* are also similar to KEYS' work. In the long run, the formula

$$W = a L^n \quad \dots\dots\dots (15)$$

is the general form, as indicated in the case of other animals, *e. g.* Molluscas (NOMURA, 1926, '28; HAMAI, 1934 a, b), Mammals (BRODY, 1927; BRODY, DAVIS and RAGSDALE, 1937), etc. The value of n is 3.04 in the

carp (MATUI, 1938), and 2.80 in summer and 3.15 in winter in the sardine, *Sardinia caerulea* (CLARK, 1934). The seasonal change in the value of n has been already confirmed by HAMAI (1935) in the Mollusca and by the above instance of *Sardinia caerulea*. As discussed above, although n is not exactly equal to 3 and is variable according to the season, in a short range of the length the formula (12) can be reasonably fitted in the weight/length relation, and, in that case, a is a variable. The formula, using f in place of a ,

$$f = 1000 \frac{W}{L^3} \quad \text{or} \quad f = 100 \frac{W}{L^3} \quad \dots \dots \dots (16)$$

is widely used as the "quality" indicator" or the "condition factor" for representing the thickness of the fish-body or the "fatness". The value of f is, then, changeable according to season, to age, and to localities. VLADYKOV (1934), CLARK (1934), AIKAWA and KATÔ (1938) and others have actually shown the variability of f in several fishes. KIMURA (1937), has examined the meaning of f and concluded from the data of *Sardinia melanosticta* as follows: "There exists

$$f = K_1 \rho c_1 c_2 \quad \dots \dots \dots (17)$$

when $v = K_1 L l' l''$, $l' = c_1 L$, $l'' = c_2 L$, where v is the volume, l' the depth, l'' the width, ρ the density and K_1 , c_1 , and c_2 the constants. f , the value of f averaged for each group, showed an increase from June to August, followed by a slight decrease in September, and another rapid increase until the mid-November. The first increase was accompanied by a diminishing density, but the second increase by a remarkable increase in the density averaged for the group. The relative fat-content in the body seems to have little effect on f . The variation in ρ , having the coefficient of variation of 0.003~0.007, cannot cause the variation in f , which the coefficient of variation is 0.03~0.06. On the other hand, the coefficient of correlation between \bar{f} and $c_1 \cdot c_2$ is so high as 0.89 on the average. The factor which governs the values of f is, thus, the thickness, but not the density of the body." SCHREBERGER (1935) has, actually calculating the values of f by means of (16), found that the values of f have nothing to do with the amount of undigested materials in the stomach-content, because there is no significant difference between the average values of f grouped into classes according to the amount of undigested materials in the stomach. It is evidently suggested, from this fact, that the variability of $c_1 \cdot c_2$ is not much affected by the variability of the amount in the stomach-content, and accordingly there is a constancy in the value

of K . If these conclusions be correct the "fatness", of special importance to the fishery industry, must be determined by the relative rate of increase in the magnitudes, PX , QY and RZ , in the writer's present species. It is already explained that the relative rate of growth in PX , QY and RZ , is the sum of the relative growth-rates of the component linear dimensions respectively. The gradient of the relative magnitude of PX , QY , and RZ was entirely in the same condition as that of X , Y and Z . And, this gradient and also this condition are almost similar to those in the volumes of the parts, V_1 , V_2 and V_3 . Therefore, to compare the thicknesses, it is enough to measure the length and width, especially Z in which a high relative growth-rate appeared in many cases. The degree of increase in thickness gradually diminishes with age, the degree of diminution in the smaller fishes being greater than that in the larger fishes. Therefore, to determine the allometric constant of width in the anal part of the body against the standard length, is a simple method to find the relative thickness of the body in a certain group of fish. Furthermore, it is necessary for the comparative use that the age is measured by the unit of physiologic age.

4. Physiologic Age

BRODY (1937) has suggested that, when the time course of growth following the major inflection can be represented by the equation,

$$\frac{W}{A} = 1 - e^{-k(t-t^*)}$$

where W is the size (*e.g.* weight) of the organism at the age t , A the size at maturity, e the base of natural logarithms, k the relative, or fractional decline in the velocity of growth with increasing age t , and t^* is the position on the age-axis where the extrapolated curve of this equation meets it, $k(t-t^*)$ will be used as unit of physiologic time, for investigations of comparative growth or senescence. It has further been suggested in the same spirit that the physiologic unit of mass should be not simple gravitational weight, but a mass as indicated by the equation

$$\text{Physiologic mass} = BW^n$$

in which n is a fractional power of the order of 0.73, and B a parameter the value of which is dependent on the unit employed. Thus, the 'physiologic age' provided by the measurements of the growth-rate should be determined by such relations as the 'physiologic time and mass' suggested by BRODY.

5. Variation in Size and the Direction-Coefficient of Variation.

It has previously been expounded that individual variation in size in a certain Mollusca can empirically be expressed by the same formula as the allometric (HAMAI, 1938). And, it has been concluded that the equation of the straight line is more accurate than the allometric equation for this purpose. In the previous chapter, it has been proved that the slope of this straight line approximately represents the ratio of the absolute growth-rates between a part and the whole, when the size is very much greater than the initial size. A similar result will be obtained regarding the nature of the allometric equation when applied to the variation of size, *i. e.* the constant α is approximately the ratio of relative growth-rates between the two, when the size is very much greater than the initial size.

When $\log x_i$ and $\log y_i$ are the increase from the initial $\log x_1$ and $\log y_1$ to the final $\log x$ and $\log y$ respectively,

$$\log x = \log x_1 + \log x_i$$

$$\log y = \log y_1 + \log y_i$$

in the same manner as in the case of the straight line. As there exists the formula,

$$\log y = \alpha' \log x + \log b'$$

for the variation in size, when only the two age-groups are considered, in which one is the 0-aged and the other is a certain n -aged group, the following relation stands for the relation between x_i and y_i :—

$$\log y_1 + \log y_i = \alpha'(\log x_1 + \log x_i) + \log b'$$

$$\frac{\log y_i}{\log x_i} = \frac{\alpha' \log x_1 + \log b' - \log y_1}{\log x_i} + \alpha'$$

When α' and $\log b'$ are finite and $\alpha' \log x_1 + \log b' - \log y_1 \neq 0$,

$$\lim_{\log x_i \rightarrow \infty} \frac{\log y_i}{\log x_i} = \alpha'$$

and also $\frac{\log y_i}{\log x_i}$ approaches α in the growth relation of the two age-groups, when $\log x_i \rightarrow \infty$. Then, α' is approximately equal to the ratio of the two relative rates of growth, when the two groups are connected by the allometric equation, or when, in other words, the growth would be attained by a constant ratio of relative growth-rates from the initial size.

But, this relation is not so accurate as the above assumption would suggest, but it is merely a rough approximation. In the degree of

accuracy, the relation derived from the straight line is certainly superior to that derived from the allometric equation, *e. g.* in general, the increase of x is much greater than that of $\log x$, compared with x , and $\log x$, respectively. Accordingly, if the condition *i. e.* that of the size being very much greater than the initial size — is not sufficiently fulfilled, the degree of accuracy becomes less. This can be proved from HAMAI's data on the Mollusca (1938).

Thus, for the calculation of the ratio of relative growth-rates, it is absolutely necessary to consider the age-factor. In the long run, the individual variation in size must be expressed by the equation of the straight line (9). This equation gives the direction of variation of the part in question against the standard part or the whole of the body. That is to say, the tangent p can be defined as the direction-coefficient of variation in a certain part against the standard part or body. Furthermore, when the increase (x_i) of the standard part or of the body is much greater than the initial size, p stands approximately for the ratio of the absolute growth-rates of the part against the standard.

VIII. SUMMARY

The growth of *Cyprinus carpio* has been studied mainly from the point of view of relative growth, and various factors affecting the growth have been analysed and brought into the following conclusions.

1) The growth curve shows the annual cycle with interruptions in the winter season. A high temperature accelerates the growth, and a low temperature slows it down.

2) The absolute growth-rate in length falls off according to advance in age, and the decreasing rate is different according to size. It is about 50% in the largest sized group, about 20% in the group of intermediate size and but little in the smallest sized group, from the 1st to 2nd year of growth.

3) The growth-rate in the period of the most intensive growth is about twice the annual average rate in the second year and about three times in the third year of growth.

4) The growth-period in the second year is longer than that in the third year, but the most intensive growth-rate is higher in the third year than in the second year.

5) The growth-rate of the larger sized group is higher than that of the smaller sized group in the anterior part of body, but in the posterior

part of body the conditions are entirely inverse, in the third year's annual rate.

6) In the period of the most intensive growth, the growth-rate of depth is greater at the middle part of the body in the smaller sized group than in the larger sized group, and that of width is greater at the head in the larger sized group than in the smaller.

7) A half amount of full diet does not allow growth to occur on average, and a less amount than this causes negative growth. The half amount of food merely keeps the maintenance of the body with no increase or decrease.

8) Individual growth shows a considerable amount of variation and is not parallel among the individuals.

9) An individual having a high growth-rate in the rapidly growing period shows a high rate always during the year, especially in the standard length, in the depths of the middle and posterior parts of the body, and in the products of depth and width (relative thickness) at the three parts of the body.

10) In the standard length, an individual having a high growth-rate in the rapidly growing period shows comparatively a high growth-rate even in the period of half-diet.

11) In the relative thickness of the posterior part of the body, the greater the size, the greater is the growth-rate in the period of full diet.

12) In the depth and in the relative thickness of the middle part of the body, the greater the size, the greater the decrease of the growth-rate in the period of half-diet.

13) In the width of the middle part, the decrease in the period of no diet is greater in larger individuals than in smaller.

14) The greater the size, the lower the relative growth-rate in the period of normal diet, in the standard length, and in the relative thickness of the middle and posterior parts of the body.

15) In the period of no diet, a correlation is found between the size of the relative thickness of the middle part of the body and its negative relative growth-rate.

16) The higher the relative growth-rate at the period of rapid growth, the higher it is during the year, in the standard length, in the relative thickness of the posterior part of the head, and in the volume of this part.

17) The negative growth in starvation is partly conditioned by the size and by the growth-activity under normal conditions.

18) The growth-centre during the period of positive growth becomes

the centre of negative growth. This growth-centre generally exists in the middle part of the body in the adult stage.

19) The relative growth-rate decreases down to a half rate from the second year to the third in annual rate.

20) The relative growth-rate of the middle part of the body only increases slightly from the second to the third year.

21) The relative growth-rate, in the period of the most intensive growth, decreases to a large extent from the first to the second year, and slightly from the second to the third, except the middle part of the body.

22) The decreasing rate of relative growth-rate is different according to size, *i. e.* that of the smaller sized fish is slow and that of the greater sized is fast.

23) The smaller the size, the younger is the physiologic age.

24) In the larval stage the growth gradient slopes from head to tail, having the growth-centre in the most anterior part of the head.

25) This growth-centre in the larval stage removes to *D*-part in the second year, and further to *C*-part in the third year. The lowest relative growth-rate is represented in the head after the second year of growth-period.

26) The physiologic age-factor affects the growth-gradient in that the relative growth-rate decreases in the head part, increases in the trunk, and increases in the width of anal part, compared with relative growth-rate of the standard length.

27) The physiologic age-factor affects the growth-gradient in the period of intensive growth in this respect — that the younger the fish the more posteriorly the growth-centre locates.

28) The growth-gradient shows a seasonal change. In the summer season, there is a tendency to a second weak growth-centre appearing in the head.

29) When the food-supply is insufficient, there is a tendency to the weak, second growth-centre appearing in the posterior part of the head, and the primary growth-centre removes posteriorly, on account of this centre changing to one of negative growth.

30) The direction of variation of a certain dimension can be expressed by the equation, $y = px + q$, against the standard length, where y is a dimension comparable to the standard length x , p is the direction-coefficient of variation, and q a constant.

31) The direction-coefficient of variation decreases with age and size,

viz. with advance in physiologic age. This tendency was found especially in the widths.

32) The direction-coefficient of variation is approximately equal to the ratio of absolute growth-rates between a dimension and the standard dimension, when the size is much greater than the initial size. Consequently, the gradient of the direction-coefficient of variation along the long-axis is almost similar to that of absolute growth-rate.

33) Although the variation is very much extended, the degree of growth arrived at in this investigation generally coincides with the growth under natural conditions.

34) It was suggested that the change of growth-gradient is subject to the growth of massive musculature which is rich in the tail and trunk.

35) The growth-data were compared with that of the other fishes, with special reference to the growth-gradient.

36) Thickness of body was discussed in relation to the increase of weight from the point of view of relative growth. In consequence, it is found that the allometry of width in the anal part of the body against the standard length determines approximately the relative thickness. As to the change in the thickness according to age and to size, it was suggested that the physiologic age must be determined.

IX REFERENCES

- AIKAWA, H. and M. KATŌ. 1938. Age Determination of Fish. I and II. Bull. Japanese Soc. Sci. Fish., 7, pp. 79-88, 89-95.
- ALLEE, W. C. 1931. Animal Aggregations. Chicago.
- BRODY, S. 1927. Growth and Development. VIII. The Relation Between Weight Growth and Linear Growth with Special Reference to Dairy Cattle. Univ. Missouri, Agricul. Exp. Stat., Res. Bull., 103.
- BRODY, S. 1937. Relativity of Physiologic Time and Physiologic Weight. Growth, 1, pp. 60-67.
- BRODY, S., H. P. DAVIS and A. C. RAGSDALE. 1937. Growth and Development. LI. Relation Between Live Weight and Chest Girth in Dairy Cattle of Unknown Age. Univ. Missouri, Agricul. Exper. Stat., Res. Bull., No. 262.
- CLARK, F. N. 1934. Maturity of the California Sardine (*Sardinia caerulea*), determined by Ova Diameter Measurements. California Fish and Game Comm., Fish. Bull., No. 42, pp. 48.
- CROZIER, W. J. and SELIG HECHT. 1913. Correlations of Weight, Length, and Other Body Measurement in the Weakfish, *Cynoscion regalis*. Bull. Bur. Fish., 33, pp. 141-147.
- DANNEVIG, A. 1933. On the Age and Growth of the Cod (*Gadus callarias* L.) from the Norwegian Skagerrack Coast. Report on Norwegian Fishery and Marine Investigations, 4, pp. 145.

- EBINA, K. 1936. On the Growth of *Pagrosomus major*. Bull. Japanese Soc. Sci. Fish., 4, pp. 411-414.
- FISHER, R. A. 1936. Statistical Methods for Research Workers. London.
- HAMAI, I. 1934 a. On the Local Variation in the Shells of *Meretrix meretrix* (L.) with Special Reference to Growth of Organism. Sci. Rept. Tōhoku Imp. Univ. Biol., 9, pp. 131-158.
- HAMAI, I. 1934 b. Relation between the Weight, Volume, and Linear Dimensions in *Meretrix meretrix* (L.). Sci. Rept. Tōhoku Imp. Univ. Biol., 9, pp. 205-212.
- HAMAI, I. 1935. On the Growth of the Shell of *Meretrix meretrix* Especially with Regard to Periodicity of Growth Relatively to the Seasonal Variation in the Environment. Sci. Rept. Tōhoku Imp. Univ. Biol., 9, pp. 339-371.
- HAMAI, I. 1937. Some Notes on Relative Growth, with Special Reference to the Growth of Limpets. Sci. Rept. Tōhoku Imp. Univ. Biol., 12, pp. 71-95.
- HAMAI, I. 1938. Relative Growth of *Meretrix meretrix* and its Local Variation, as Shown by Experiments in Rearing. Sci. Rept. Tōhoku Imp. Univ. Biol., 13, pp. 205-220.
- HAMMETT, F. S. and D. W. HAMMETT. 1939. Proportional Length Growth of Gar (*Lepisosteus platyrhincus* DE KAY). Growth, 3, pp. 197-209.
- HECHT, SELIG. 1913. The Relation of Weight to Length in the Smooth Dog Fish, *Mustelus canis*. Anat. Rec., 7, pp. 39-42.
- HECHT, SELIG. 1916. Form and Growth in Fishes. Journ. Morphol., 27, pp. 379-400.
- HUXLEY, J. S. 1932. Problems of Relative Growth. London.
- HUXLEY, J. S. and TEISHER, G. 1936. Terminology of Relative Growth. Nature, 137, pp. 780.
- KATOH, G. 1932. On the Sex Ratio and the Growth of Body in *Carassius auratus* and its Variety "the Iron-Fish". Sci. Rept. Tohoku Imp. Univ. Biol., 7, pp. 365-381.
- KEARNEY, H. L. 1914. On the Relative Growth of the Organs and Parts of the Embryonic and Young Dogfish (*Mustelus canis*). Anat. Rec., 8, pp. 271-297.
- KIMURA, K. 1937. Thickness and Density of the Fish Body. Bull. Japanese Soc. Sci. Fish., 6, pp. 69-72.
- KUROKI, EIITI. 1939. Carps (*Cyprinus carpio* L.) and Crucians (*Carassius auratus* (L.)) from the Lakes of Aoki, Nakatuna and Kizaki, Nagano Prefecture. Bull. Japanese Soc. Sci. Fish., 8, pp. 69-72.
- MATSUBARA, KIYOMASU. 1935. Studies of the Scorpaenoid Fishes of Japan. II. Statistical Observations on *Sebastodes innermis* (C. et V.). Bull. Japanese Soc. Sci. Fish., 4, pp. 217-223.
- MATUI, I. 1938. Suisan Seibutu no Seityōdo no Kenkyū. IV. Ko-ka Gyorui no Nenrei to Taityo oyobi Taizyu tonō Kankei. Studies of the Growth-Rate in Aquatic Animals. IV. The Relation between the Length, Weight and the Age in *Cyprinidae*. Dōbutugaku Zasshi (Zool. Mag. Tōkyō), 50, pp. 184-185.
- MOTILEY, MAC. 1936. A Biometrical Study of the Kamloops Trout of Kootenay Lake, *Salmo kamloops* JORDAN. Journ. Biol. Board. Canada, 2, (4).
- NEEDHAM, A. E. 1935. On Relative Growth in the Jaws of Certain Fishes. Proc. Zool. Soc. London, 1935, pp. 773-784.
- NOMURA, E. 1926. An Application of $a-kb^c$ in Expressing the Growth Relation in the Freshwater Bivalve, *Sphaerium heterodon* PUS. Sci. Rept. Tohoku Imp. Univ. Biol., 2, pp. 57-62.
- NOMURA, E. 1928. On the Relation between Weight and Dimensions in the Bivalves, *Tapes philippinarum* and *Cytherea meretrix*. Sci. Rept. Tōhoku Imp. Univ. Biol., 3, pp. 113-124.

- PAULSEN, E. M. 1933. Biology of the Dab in Danish Waters. Rept. Danish Biol. Stat., 38, pp. 9-30.
- SCHNEBERGER, E. 1935. Growth of the Yellow Perch (*Perca flavescens* MITCHELL) in Nebish, Silber and Weber Lakes, Vilas County, Wisconsin. Trans. Wisconsin Acad. Sci., Arts and Letters, 29, pp. 103-130.
- THOMPSON, D'ARCY W. 1917. On Growth and Form. Cambridge.
- VLADYKOV, V. D. 1934. Young Haddock in the Vicinity of Halifax. Contr. Canad. Biol. Fish., N. S., 8, pp. 411-419.
- WEYMOUTH, F. W. and H. C. McMILLIN. 1930. Relative Growth and Mortality of Pacific Razor Clam (*Siliqua patula* DIXON) and their Bearing on the Commercial Fishery. Bull. Bur. Fish., 46, pp. 543-567.
- WEYMOUTH, F. W. and S. H. THOMPSON. 1930. The Age and Growth of the Pacific Cockle (*Cardium corbis* MARTYN). Bull. Bur. Fish., 46, pp. 633-641.
- WIELENBACH, E. P. 1938. Neue Wege der Karpfen Züchtung. Arbeit. Biol. Versuchsanst. München, No. 150, pp. 1-19.

EMBRYOGENY OF *PODOCARPUS MACROPHYLLUS* AND *PODOCARPUS NAGI**

By

MASATO TAHARA

Biological Institute, Tôhoku Imperial University, Sendai

(With Plates II, III and 12 text-figures)

(Received October 29, 1940)

The genus *Podocarpus*, including two subgenera *Stachycarpus* and *Protopodocarpus*, contains about 60 species. These are distributed mostly in the Southern Hemisphere. But two species of *Protopodocarpus* grow in Japan, namely *P. macrophyllus* and *P. Nagi*. These two are quite different in external appearance, the former belonging to the section *Eupodocarpus* and the latter to the section *Nageia*. The embryogeny of these two plants will be described in the present paper.

In 1902 COKER published a paper on the gametophyte and embryo of *Podocarpus coriacea*, an American species belonging to *Eupodocarpus*. In 1912 STILES and in 1913 SINNOTT gave accounts regarding the gametophyte and embryo of a number of species of *Podocarpus*. More recently (1936) BUCHHOLZ has described the embryogeny of three species of the subgenus *Stachycarpus*. But up to the present our knowledge concerning the embryogeny of the plants belonging to the section *Nageia* of the subgenus *Protopodocarpus* remains very incomplete.

The materials of the present investigation were collected mostly in the summer of this year (1940) in several places in Kanagawa Prefecture. They were fixed with Navashin's fluid after dipping them in alcohol-acetic-formalin for ten minutes.

At this point the writer wishes to take the opportunity to thank Mr. T. SHIMAMURA and Mr. T. KOSHIMIZU for their valuable assistance in securing the material.

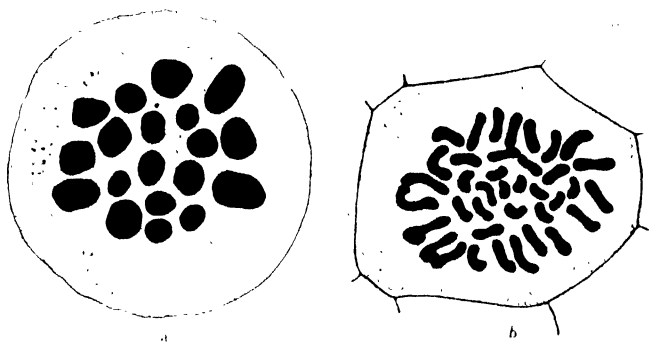
1. *Podocarpus macrophyllus*

In Japan this plant grows wild and is commonly cultivated as a garden plant. The somatic chromosome number of this plant was originally in-

* This investigation was carried out under the expence of the Kagaku-Kenkyuhi of the Department of Education, Japan.

vestigated by FLORY (1936). He states: "The number is about 38, many plates seeming to have this count. In several cases there was a suggestion of 39 chromosomes. No plate seems to have 40 but perhaps, for safety, the number should be placed as between 38 and 40".

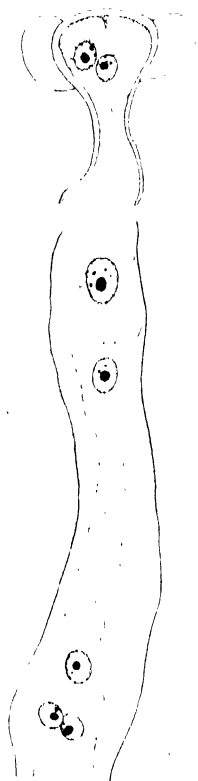
The present writer investigated the haploid chromosome number in the 1st meiotic division of the pollen mother cells by acetocarmin method and estimated it to be 19 (Text-fig. 1, a). Text-fig. 1, b is a polar view of the metaphase of a mitosis in a cell of the root tip of a seedling of this plant. Here 38 chromosomes are counted.



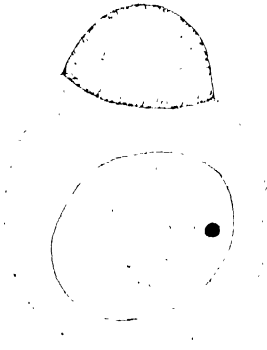
Text-fig. 1. *P. macrophyllus* a, 1st meiotic division in a pollen mother cell. b, mitosis in a root tip. $\times 1650$.

Fertilization of this plant occurs in the middle of July. In the pollen tube penetrating the nucellus a number of free nuclei is observed, as is usual in the species of *Podocarpus* (Text-fig. 2). Mixed with these free nuclei the body-cell nucleus, embedded in dense cytoplasm, is rather conspicuous. Just before fertilization this nucleus divides into two sperm nuclei, of which the non-functional one is smaller and naked, as already reported by STILES (1912) for this plant.

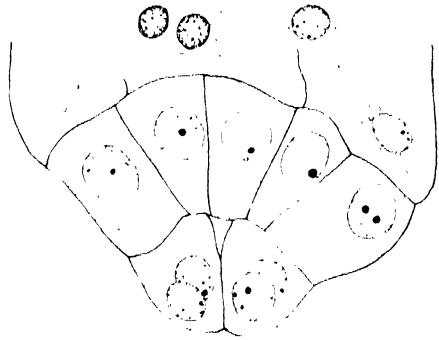
Unfortunately the act of fertilization was not observed. Two nuclei resulting from the 1st sporophytic nuclear division still remain in the middle portion of the archegonium. Migration of nuclei to the bottom of the archegonium occurs in the 4-nucleus stage. Two successive divisions of these 4-nuclei give rise to the 16-nucleus stage. By these divisions the nuclei become smaller and smaller (Pl. II, Fig. 1 6). Cell wall formation initiates in the 16 nucleus stage,



Text-fig. 2. *P. macrophyllus*. Germination of a microspore. $\times 360$.

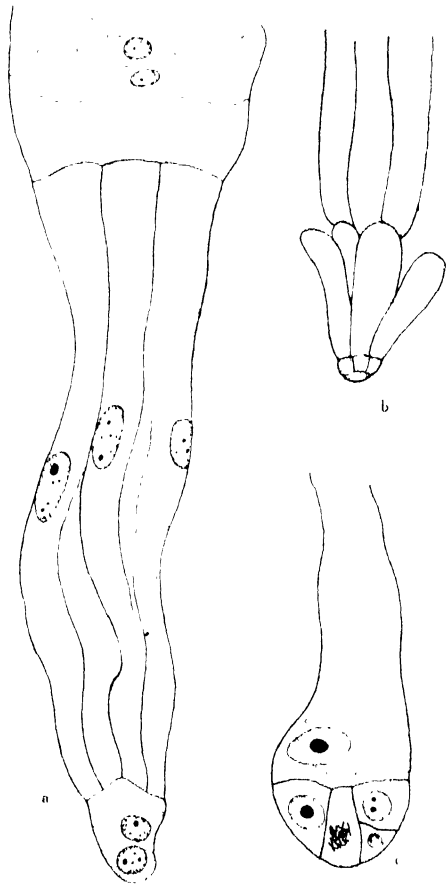


Text-fig. 3. *P. macrophyllus*. Two sperm nuclei produced by the division of a body cell nucleus. $\times 480$.



Text-fig. 4. *P. macrophyllus*. Proembryo having two tip cells. $\times 470$.

proembryo of two or three tiers being thus formed. The terminal tier is composed of a single tip cell, containing two free nuclei. Only in one case two tip cells, each containing two nuclei, were observed (Text-fig. 4). The second tier forms the suspensor, the number of the cells in this tier varying perhaps from 8 to 14. When 14 nuclei are used in the formation of this tier, the third or rosette tier is not formed at all (Pl. II. Fig. 9), or the rosette tier is formed by the division of the nuclei of the second tier. In the latter case the second tier has at first no wall towards the general cytoplasm of the archegonium (Pl. II. Fig. 7). In the case, where less than 14 nuclei are used in the formation of the second tier, the rosette tier is formed at the same time as the second tier,



Text-fig. 5. *P. macrophyllus*. Different stages of embryonal development. $\times 360$.

without the nuclear division in the second tier (Pl. II. Fig. 8).

In passing it may be worthy of mention that a number of free nuclei, perhaps originating from a pollen tube, are found often in the basal portion of an archegonium and it is likely that they may be mistaken for rosette nuclei. Two nuclei above the proembryo shown in Text-fig. 5, a, for example, cannot be decided therefore at once as being rosette nuclei.

Later the suspensor elongates to an enormous length and a single embryo is formed at the end of the suspensor. Thus the regular occurrence of cleavage polyembryogeny is not observed in the normal course of development of this plant. But it is not rare that the cells constituting a suspensor become separated from each other and an embryo is formed at the end of each single celled suspensor (Text-fig. 5, c).

As already observed in the other species of *Podocarpus* (COKER, 1902) a thick plug formed at the upper end of the suspensor is very conspicuous in the later stage of embryonal development.

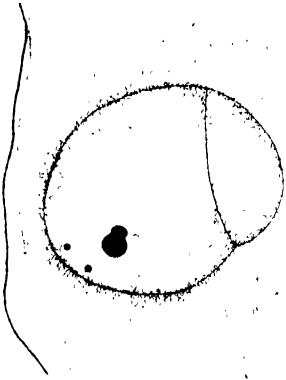
The endosperm cell of this plant has naturally at first one nucleus. But later those surrounding the embryo become multinucleate.

2. *Podocarpus Nagi*

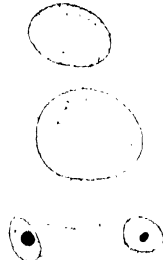
Podocarpus Nagi is one of the most beautiful conifers in Japan and is commonly cultivated as an ornamental tree in the warmer regions of this country. The material of this plant was collected in the summer of this year (1940) in a private garden in Odawara, Kanagawa Prefecture. Unfortunately the material for the meiotic division in pollen mother cells could not be obtained.

Fertilization of this plant takes place also in the middle of July (Textfig. 6). The body-cell found near the end of a pollen tube is spherical in shape and is surrounded by a number of small nuclei. One of the two sperm nuclei resulting from the division of the body-cell nucleus is situated at one extremity of the body-cell, while the other is found in the centre of the cell, as already noted by STILES (1912) in describing this plant (Text-fig. 7).

After fertilization the fusion nucleus divides into two. They still remain in the middle part of the archegonium. But the nuclei in the next four nucleus stages are found at the bottom of the archegonium. The eight, sixteen and thirty two nucleus stages come next (Pl. III). Cell formation occurs in the 32 nucleus stage. The cells, each containing a single nucleus are arranged in two tiers (Text-fig. 10). The cells of



Text-fig. 6. *P. Nagi*. Fertilization. (From the material collected on July 12.) $\times 630$.

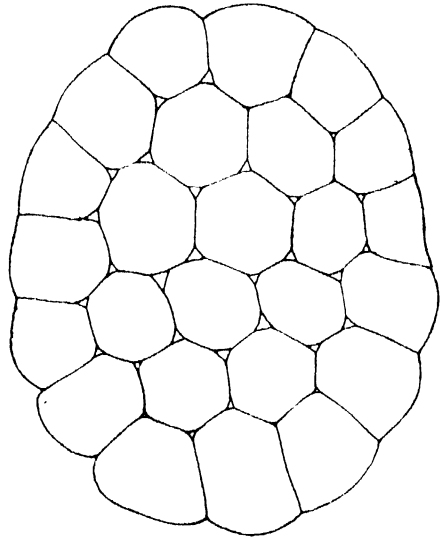


Text-fig. 7. *P. Nagi*. Sperm nuclei. Two small nuclei shown in lower position are the pollen tube nucleus and the stalk cell nucleus. $\times 480$.



Text-fig. 8. *P. Nagi*. 1st division of the fusion nucleus. $\times 630$.

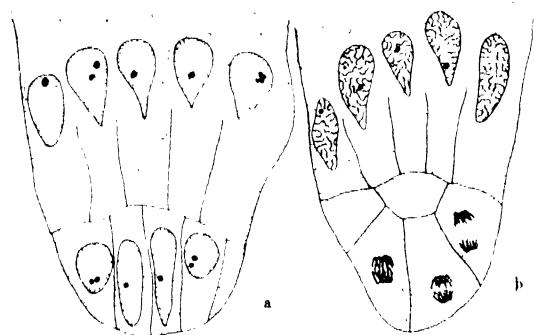
the lower tier, seven to nine in number, are the embryonic initial units. By the next nuclear division they all become binucleate (Pl. III, Fig. 18). The cells in the upper tier, 23 to 25 in number, have no wall toward the general cytoplasm of the archegonium. But the next nuclear division, which comes a little later than that in the lower tier, produces prosuspensor cells and free rosette nuclei. For some time elongation of the prosuspensor continues and in early August divisions of the embryonic initial cells begin, and separate embryos having no primary suspensor are produced clustered at the end of the prosuspensor (Text-fig. 11 and 12). Thus cleavage polyembryony is a regular occurrence in the embryogeny of this plant.



Text-fig. 9. *P. Nagi*. Cross section of a prosuspensor, consisting of 25 cells. Cross section of the terminal portion of the same embryo is shown in Fig. 17, Pl. III. $\times 470$.

BUCHHOLZ (1932) reports cleavage polyembryony in a species of *Dacrydium*, a genus closely related to the genus *Podocarpus*. In this

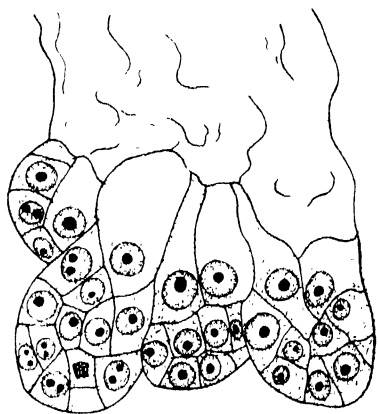
plant the embryo situated in the terminal portion appears to be more favorable for development than the remaining ones, and is ordinarily destined to become the successful embryo. BUCHHOLZ' name for this type of cleavage polyembryony is "determinate" cleavage polyembryony. The type of embryogeny in *Podocarpus Nagi* seems to be "in-



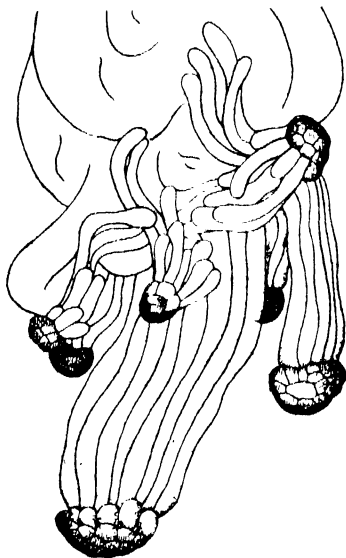
Text-fig. 10. *P. Nagi*. a, cell formation in 32 nucleus stage. The cells are arranged in two tiers. b, sixth mitosis. $\times 470$.

determinate" cleavage polyembryony.

According to a more recent investigation of BUCHHOLZ (1936) three *Podocarpus* species belonging to the subgenus *Stachycarpus* have seven to twelve binucleate embryonic cell units at the end of a prosuspensor. These units, however, gives rise to



Text-fig. 11. *P. Nagi*. Separate embryos produced at the end of a prosuspensor. Younger stage. $\times 360$.



Text-fig. 12. *P. Nagi*. Separate embryos produced at the end of a prosuspensor. Later stage. Each embryo has already formed embryonal tubes. $\times 90$.

groups of cells, which usually combine to form a single embryo. No regular occurrence of cleavage polyembryony has been known of before in the genus *Podocarpus*. Its existence in *Podocarpus Nagi* therefore must be considered as a remarkable characteristic of this plant.

Generally in *Podocarpus*, after fertilization, four successive nuclear divisions occur in the egg-cell and 16 nuclei are produced before the cell-formation. Strikingly in *Podocarpus Nagi* five successive nuclear divisions take place before the cell-formation, 32 nuclei being arranged in two tiers and by the next sixth nuclear division 64 nuclei are produced. Similar cases are found only in *Agathis*, *Araucaria* and *Sciadopitys*.

SUMMARY

1. In *Podocarpus macrophyllus* the fusion nucleus repeats four successive nuclear divisions and produces sixteen free nuclei. Cell wall formation begins in this sixteen nucleus stage. Only one binucleate tip cell is formed at the end of the prosuspensor. Cleavage polyembryony is, however, sometimes observed also in this plant.

2. In *Podocarpus Nagi* the fusion nucleus carries out five successive nuclear divisions. The thirty two nuclei resulting from these divisions are arranged in two tiers. By the sixth nuclear division the cells of the lower tier, seven to nine in number, become binucleate, and the cells of the upper tier, 23 to 25 in number, segregate free rosette nuclei, which soon become degenerate. Cleavage polyembryony regularly occurs in this plant.

LITERATURE

- BUCHHOLZ, J. T. 1933. Determinate Cleavage Polyembryony, with Special Reference to *Dacrydium*. Bot. Gaz. 94.
- BUCHHOLZ, J. T. 1936. Embryogeny of Species of *Podocarpus* of the Subgenus *Stachycarpus*. Bot. Gaz. 98.
- BURLINGAME, L. L. 1914. The Morphology of *Araucaria brasiliensis*. II. The Ovulate Cone and the Female Gametophyte. Bot. Gaz. 57.
- COKER, W. C. 1902. Notes on the Gametophytes and Embryo of *Podocarpus*. Bot. Gaz. 33.
- EAMES, A. J. 1913. The Morphology of *Agathis australis*. Ann. Bot. 27.
- FLORY, W. S. 1936. Chromosome Numbers and Phylogeny in the Gymnosperm. Jour. Arnold Arb. 17.
- SINNOTT, E. W. 1913. The Morphology of the Reproductive Structure in the Podocarpaceae. Ann. Bot. 27.
- STILE, W. S. 1912. The Podocarpeae. Ann. Bot. 26.
- TAHARA, M. 1940. The Gametophytes, Fertilization and Proembryo of *Sciadopitys verticillata*. Sci. Rep. Tôhoku Imp. Univ. 15.

EXPLANATION OF PL. II.

Podocarpus macrophyllus.

Fig. 1. Egg nucleus. Fig. 2. Two nucleus stage. Fig. 3. Four nucleus stage. Fig. 4. Eight nucleus stage. Five smaller nuclei shown in upper position are perhaps the ones brought by a pollen tube. Fig. 5. Fourth mitosis to give rise to sixteen nucleus stage. Fig. 6. Sixteen nucleus stage. Fig. 7. Sixteen nuclei are arranged in two tiers. Mitosis occurs in the upper tier. Two nuclei shown in upper position are perhaps the ones brought by a pollen tube. Fig. 8. Proembryo consisted of cells arranged in three tiers. Fig. 9. Proembryo consisted of cells arranged only in two tiers. Magnification: $\times 470$.

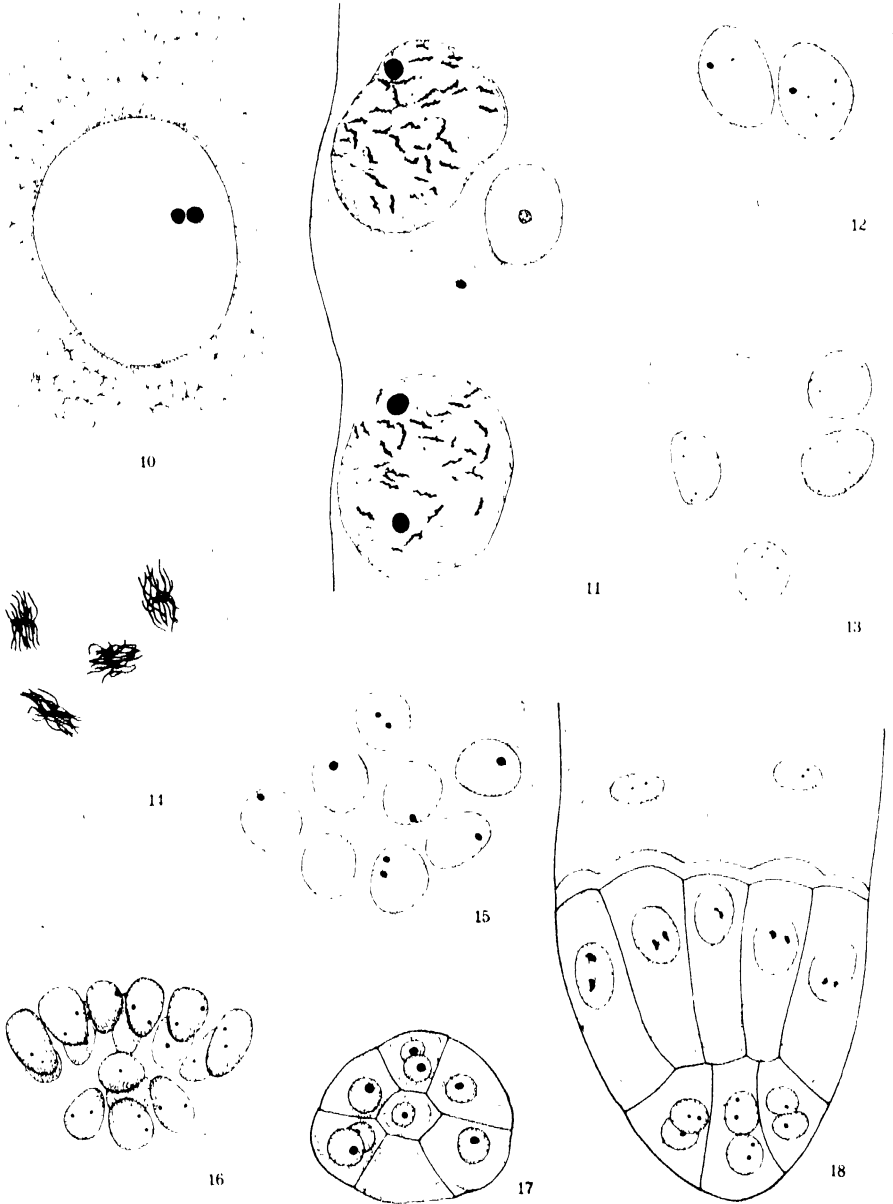
EXPLANATION OF PL. III.

Podocarpus Nagi.

Fig. 10. Egg nucleus. Fig. 11. Two nucleus stage. Fig. 12. Four nucleus stage. Fig. 13. Eight nucleus stage. Fig. 14. Fourth mitosis to give rise to sixteen nucleus stage. Fig. 15. Sixteen nucleus stage. Fig. 16. Thirty two nucleus stage. Fig. 17. Cross section through terminal portion of a proembryo. Fig. 18. Finished proembryo. Magnification: $\times 470$.



M. TAHARA: *Podocarpus macrophyllus*



THE GAMETOGENESIS, THE BREEDING HABITS, AND THE EARLY DEVELOPMENT OF *ARENICOLA CRISTATA* STIMPSON, A TUBICOLOUS POLYCHAETE¹

BY

KATSUHIRO OKADA

Biological Institute, Tôhoku Imperial University, Sendai

(With Plates IV-VI and 25 text-figures)

Received November 5, 1940

CONTENTS

Introduction	98
Material and Methods	100
The Gametogenesis	101
The Spermatogenesis	
The Oögenesis	
The Maturing Season of Gametes	
The Breeding Habits	107
Experiments on the Discharge of Generative Products and the Fertilization	110
Remarks on the Egg-mass Forming Habits	116
The Early Development	117
The Cleavage	
The Gastrulation	
The Early Larval Stages	
Discussion	133
Summary	142
References	144

INTRODUCTION

In spite of the world-wide distribution and the frequent local abundance of the polychaetes belonging to the family Arenicolidae, only a few writings have so far been published concerning their embryology. In these papers, we have no complete account of and very little information about the embryology of the Arenicolidae. In the present paper, the writer will give a detailed account of the gametogenesis, the breeding habits and the early development of a common Japanese species of *Arenicola*, found near the Asamusi Marine Biological Station. This

¹ Contribution from the Marine Biological Station Asamushi, Aomori-Ken. No. 172

embryological and ecological study was undertaken at the suggestion of Prof. Dr. E. NOMURA, and was carried out in the breeding seasons of 1938 and 1939. The writer has, here, to express his grateful thanks for the kind guidance received from Prof. E. NOMURA during this investigation.

MATERIAL AND METHODS

The animal used in the present study was classified as *Arenicola cristata* STIMPSON by the kind identification, made by Mr. KEIZO TAKAHASHI. It is found abundantly in the littoral zone of Moura sea-shore, where the coastline of Mutu Bay curves and forms a calm inlet about four miles north of the Asamusi Marine Biological Station, Aomori Prefecture. The latitude of this habitat is 40° 53' north. It burrows into the sandy bottom and is characterized by its sand-castings (Fig. 1). The burrows are usually



Fig. 1. Photograph, showing sand-castings and egg-masses of *Arenicola cristata* in aquarium. $\times 1/6$.

U-shaped. One end of the burrow is the head-opening where the sand becomes hollow in a shallow funnel-like depression, and the other is the tail-opening where the casting is piled up on the surface of the sandy bottom. The worm lies almost invariably with the dorsal side downwards inside the burrow.

The embryological observation was carried out of both live and fixed specimens. For fixation, the specimens were killed in Bouin's solution. The sections were made serially, 10 μ or 15 μ in thickness, by the paraffin method, and stained chiefly by Mallory's connective tissue staining method.

* It is, here, worthy of note that the present investigation was carried out at the expense of Kagakukenyuhi of the Department of Education, Imperial Japanese Government.

For the purpose of the ecological investigation, in which the breeding habits and the related environmental factors were studied, standard stations S_1 and S_2 were provided in the habitat (Fig. 2). The area of S_1 was 10 m.² in the water in the shallower portion of the habitat, where the depth measured about 0.5 m. at the higher high water and the bottom was dry at the lower low water of the spring tides during the breeding season. The area of S_2 was 35 m.² in the deeper portion of the habitat, where the depth measured about 1.2 m. at the higher high water and the bottom was never dry even at the lower low water of the spring tides during the breeding season. Besides the investigations at these standard stations, a few rearing methods were practised experimentally in glass vats, in wire cages and in the aquaria of the Asamusi Marine Biological Station. The glass vats used in this experiment were 35 cm. long, 25 cm. wide and 30 cm. high. The wire cages were used when it



Fig. 2. General view of Moura sea-shore. (A) set of standard station S_1 , (B) same of standard station S_2 , (C) same of isolated rearing.

was desired to isolate the animal in the habitat (Fig. 2). They were 75 cm. long, 60 cm. wide and 50 cm. high. In the aquarium, which was 60 cm. long, 55 cm. wide and 75 cm. high, sand from the habitat was laid on the bottom at a thickness of about 20 cm., and sea-water continually being renewed was at a constant depth of about 0.5 m.

THE GAMETOGENESIS

The sexes are separate in *Arenicola cristata*. The reproductive organ is constituted of six pairs of gonads without any accessory organ and is closely associated with nephridia. The gametogenesis of this animal, therefore, will be described in the following order first the spermatogenesis,

secondly the oögenesis, and then the maturing season of the gametes.

THE SPERMATOGENESIS.

In the testes, several young spermatogonia group together to form a spherical mass called the spermatophore. These masses of spermatogonia are liberated from the testes into the coelom. In the coelomic fluid, the spermatophores undergo the spermatogenesis without disintegrating the mass-formation and develop into masses of the ripe spermatozoa. Accordingly, countless spermatophores in various spermatogenetic stages are easily found in the coelom of the mature male.

The youngest spermatophore found in the coelomic fluid is usually constituted of ten or more spermatogonia of early generation and assumes a morula-like appearance. The nucleus of each spermatogonium, in the fixed condition, is spherical, measures about 7μ in diameter, and shows rather a clear aspect with a few chromatin, sometimes a small nucleolus appearing. Its cytoplasm is of small quantity but comparatively distinct (Fig. 3 A). Such a young spermatophore grows gradually by repeating several divisions of its constituents. Therefore, the nucleus of each spermatogonium of the later generation measures about 5μ in diameter, and contains more chromatin than that in the early generation, no nucleolus appearing in most cases (Fig. 3 B).

Thus, the last generation of the spermatogonial mass enters the early generation of the spermatocytal mass. In this spermatocytal mass-formation, each spermatocyte undergoes meiosis. It is difficult, however, to observe precisely the details of the meiosis, because of the minuteness of its karyokinetic figures. The only supposed stages of the synapsis and of the interkinesis can be found among these figures (Fig. 3 C & D). After the meiosis, each spermatid assumes an oval shape and measures about $3\mu \times 2\mu$ in long and short diameters respectively. In the fixed condition, its nucleus is a compact irregular mass. By the posterior side of the nucleus, one or two clear granules are always present in its cytoplasm (Fig. 3 E & F). Meanwhile, each spermatid enters the period of spermioteleosis. In the process of spermioteleosis, the spherical mass of spermatids transforms into a disc-like mass which may be called the sperm-disc (Fig. 3 G). In this sperm-disc, the head of each spermatozoön is directed towards the centre and the tail towards the exterior (Fig. 3 H). The size of these discs varies with the number of their constituents. Most of these discs measure about 40μ in diameter and about 12μ in thickness.

After their discharge into the sea-water, the ripe sperm-disc begins to disintegrate and many spermatozoa swim away separately (Fig. 3 I). Each ripe spermatozoon consists of a club-shaped head and a long tail. The head measures about 1μ in length. Its anterior end is slender and may represent the acrosome. Its posterior end is thicker and may contain the middle piece, a somewhat clear point being present here. The remaining main portion is occupied by the nucleus staining with increasing intensity. The tail appears finely attenuate and is about ten times as long as the head (Fig. 3 J).

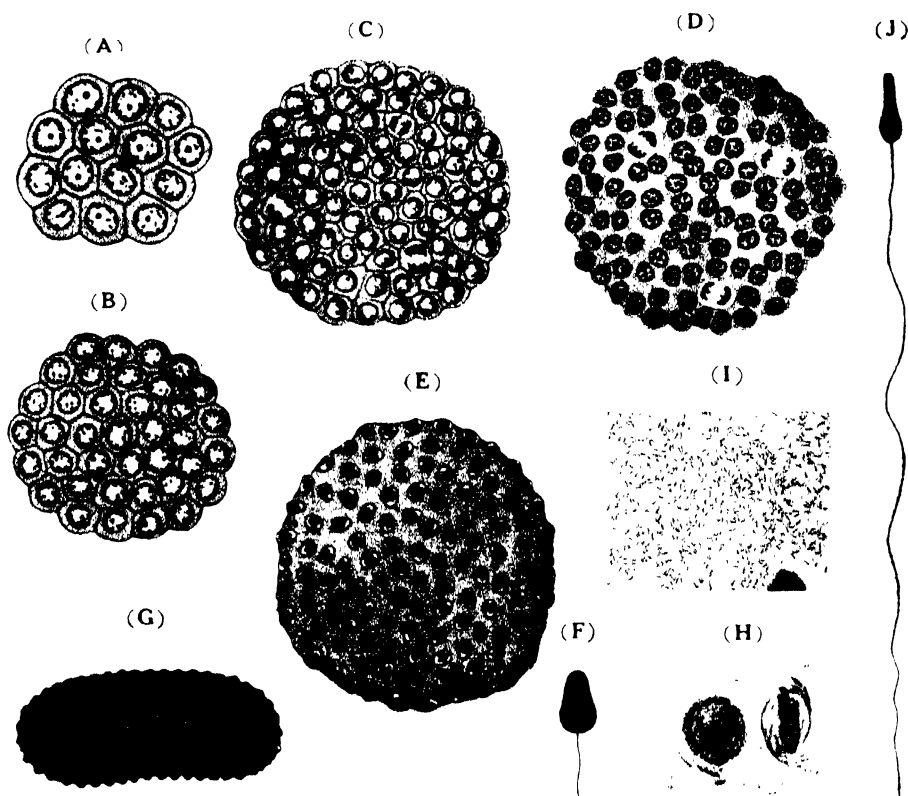


Fig. 3. Stages of spermatogenesis. (A) youngest spermatophore constituted of spermatogonia of early generation. $\times 750$. (B) young spermatophore constituted of spermatogonia of later generation. $\times 750$. (C) spermatophore constituted of primary spermatocytes. $\times 750$. (D) spermatophore constituted of secondary spermatocytes. $\times 750$. (E) spermatophore constituted of spermatids. $\times 750$. (F) spermatid. $\times 2000$. (G) ripe sperm-disc of spermatozoa. $\times 750$. (H) photomicrograph of two ripe sperm-discs in frontal and side views respectively. $\times 200$. (I) photomicrograph of spermatozoa disintegrated from mass-formation. $\times 200$. (J) spermatozoon. $\times 2000$.

THE OÖGENESIS.

The ovarian eggs produced by the multiplication of oögonia are liberated freely from the ovary into the coelom, when they have reached a diameter of about 15μ . After liberations, these young oöcytes are floating in the coelomic fluid and grow to be primary oöcytes.

During the growth period of each oöcyte, the nucleus becomes more vesicular and the oöplasm is increased extensively by the deposition of the

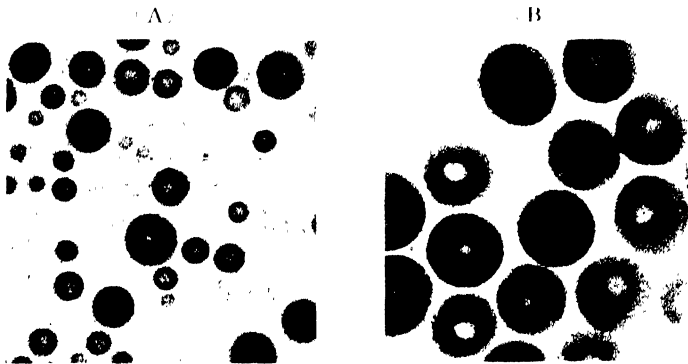


Fig. 4. Photomicrographs, showing polar view of live ova in coelom. $\times 70$
(A) young oöcytes in growth period (B) primary oöcytes.

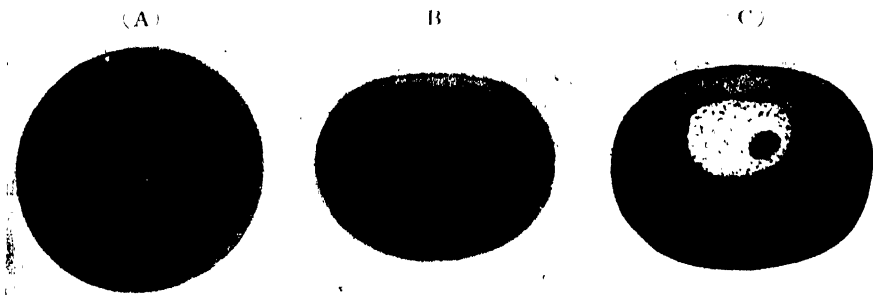


Fig. 5. Photomicrographs, showing polar view (A) and side view (B) of live primary oöcytes in higher magnification, and illustration of fixed primary oöcyte in vertical section (C). $\times 230$.

deutoplasm. These growing oöcytes are discoïdal in shape. The increasing deutoplasm is distributed somewhat abundantly at the portion near the nucleus. And, the growth of the oöplasm is more remarkable than that of the nucleus. Therefore, the nucleoplasmatic ratio of a given oöcyte decreases with the advance of its growth (Fig. 4). In the grown primary oöcyte, the two diameters in the frontal view are about equal to each other and measure 150μ or so, while the third diameter in the side view

measures about $120\ \mu$ (Fig. 5 A & B). This third axis coincides with the future polar axis of the mature ovum. The oöplasm of the primary oöcyte, surrounded by a distinct vitelline membrane, becomes somewhat homolecithal and opaque, owing to a brownish-violet pigments contained in the deutoplasm. The so-called germinal vesicle with a germinal spot lies a little eccentrically towards the animal pole (Fig. 5 C).

These oöcytes fully grown are discharged into the sea-water outside the body and are wrapped in a jelly-like substance. In each discharged oöcyte, the germinal vesicle rapidly disappears, and the signs of the first maturation division begin to appear at the region near the animal pole. At first, a spindle is formed about parallel to a tangential plane (Fig. 6 A), and then it rotates through ninety degrees, putting its axis in a radial position. During this rotation, three or more asters are usually found in this karyokinetic figure (Fig. 6 B). And the extra-asters invariably vanish when the rotation of the spindle is completed. In such a metaphase of the first polar division, the maturation process of each discharged oöcyte is not completed-till the actual entrance of the spermatozoön (Fig. 6 C).

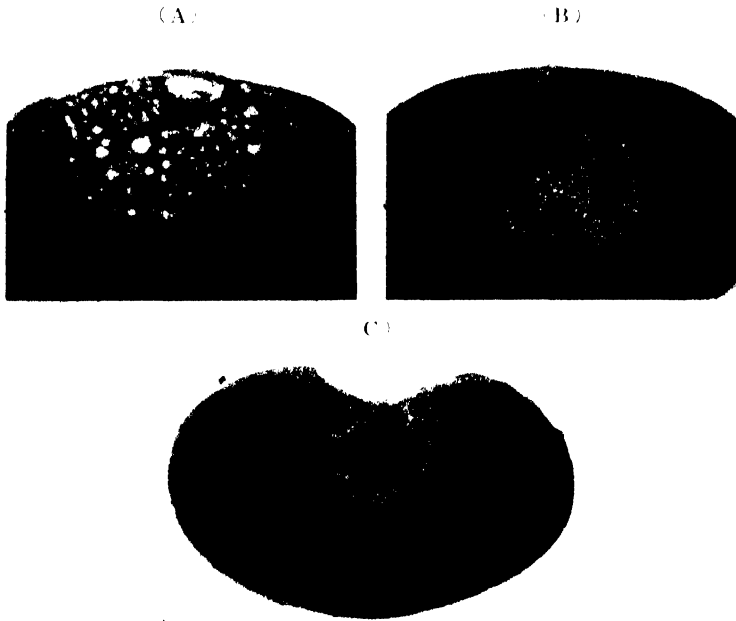


Fig. 6 Photomicrographs of maturing primary oocytes. (A) vertical section, showing disappearance of germinal vesicle and tangential position of first polar spindle. $\times 550$. (B) same, showing rotation of first polar spindle and extra-aster formation. $\times 550$. (C) same, showing metaphase of first polar division. $\times 370$.

THE MATURATING SEASON OF GAMETES.

The spawning season of *Arenicola cristata* at Moura begins about the middle of July and continues with variations of activity until the middle of September. For example, the first egg-masses laid in 1938 were found on 20th July, and those in 1939 were found on 11th July, and the last egg-masses laid in 1939 were found on 19th September, and those in 1940 were found on 11th September. The maturing season of the gametes begins a little previously to, and covers this spawning season.

In the specimens collected at the beginning of June, the generative products in either sex were not yet liberated into the coelomic fluid. The inner organizations of these immature worms can be learned to some degree through the body-wall. Their bodies were also not fully grown. In most specimens collected at the latter part of June, the generative products had begun to be liberated from the gonads into the coelom. About this time, the ova obtained from the coelom of the female are in the process of the growth period, and no ripe sperm-disc can be yet obtained from the coelom of the male. At the beginning of July, the gametogenesis in either sex becomes suddenly active, and the body cavity is filling with the generative products. Meanwhile, the external discrimination between the male and the female becomes easy on account of the numerous generative products in their coeloms. In aggregation the colour of the spermatophores of this species is white, that of the body-wall in each mature male being creamy from the outside view (Pl. VI, Fig. 31). The oöplasm is brownish-violet as stated already, and the colour of the body wall in each mature female becomes brownish-pink from the outside view (Pl. VI, Fig. 32). About the middle of July, both the male and the female are fully grown and their gametogeneses acquire maximum activity. Henceforth, this maximum activity of the gametogenesis in either sex continues for a month or more, while the ripe generative products are discharged outside the body. In September, the gametogenesis gradually lessens on the approach of the closure of the spawning season.

On the other hand, varying rates of growth are shown in the specimens by divisions even in a given habitat (Table 1). Among the fully mature specimens which can discharge their generative products, the entire length of the largest specimen is about 30 cm. in an extended condition, and its casting-cord is about 6 mm. in thickness, while, the smallest one is about 15 cm. and its casting-cord about 3 mm.

TABLE 1. The population of *Arenicola cristata* in the standard stations S₁ and S₂ during July of 1939. The growth rate of the specimens was indicated by thickness of the casting-cord, and all the specimens investigated were fully matured on 25th July.

Date and station of investigation.	Population of specimens per 1m. ²	Percentage of specimens measured by thickness of casting-cord.			
		6-5 mm.	5-4 mm.	4-3 mm.	3-2 mm.
6th July { S ₁	3.1 ± 0.8	—	26%	65%	9%
	S ₂ 11.2 ± 2.2	—	7%	49%	44%
14th July { S ₁	2.7 ± 0.8	18%	81%	1%	—
	S ₂ 11.6 ± 2.5	—	14%	77%	9%
25th July { S ₁	3.2 ± 1.1	56%	44%	—	—
	S ₂ 11.5 ± 2.2	21%	61%	18%	—

THE BREEDING HABITS

It is presumed, in connection with anatomical information, that the fertilization occurs externally. In fact, the spermatozoa and the ova are discharged respectively outside the body before the fertilization. In the natural habitat, however, the actual process of the discharging of the generative products in either sex is obscure, because of its occurrence inside the burrow in the sandy bottom. Accordingly, it can only be observed by keeping the animals in glass vats without sand.

In the male, the ripe sperm-discs are discharged slowly by a series of peristaltic movements of the body through six pairs of nephridia, which play the actual part of sperm-ducts. These sperm-discs are wrapped in a jelly-like substance secreted from the surface of the body, and disintegrate at once into numerous spermatozoa. In the female, the ripe ova are also discharged through the nephridia, which play the actual part of oviducts. In this case, a greater amount of jelly-like substance is secreted wrapping the discharged ova. These discharged ova are fertilized before long inside the burrow by the invading spermatozoa.

The actual processes of the fertilization are observed by means of artificial insemination of the ripe ova. With the entrance of spermatozoön, the fertilization membrane in each ovum is first lifted (Fig. 7 A), and its oöplasm becomes somewhat yellowish. The entrance of spermatozoön into each ovum occurs always at the metaphase of its first polar division (Fig. 7 B & C). The spermatozoön entering at any point of the vegetal hemisphere of the ovum moves towards the animal pole (Fig. 7 D & E), while,

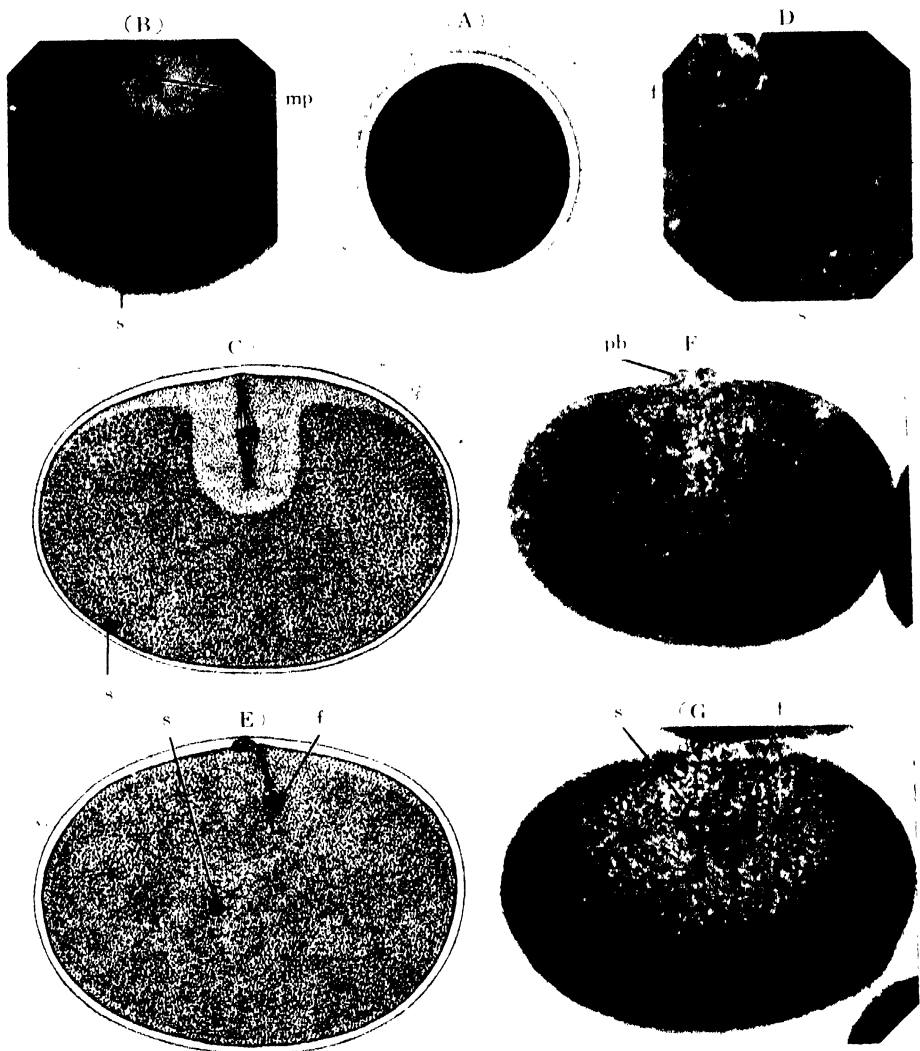


Fig. 7. Processes of fertilization. (A) photomicrograph, showing polar view of live fertilized ovum and lifting of fertilization membrane. $\times 200$. (B) photomicrograph, showing entrance of spermatozoön into ovum. $\times 370$. (C) scheme, showing vertical section of ovum immediately after entrance of spermatozoön. $\times 370$. (D) photomicrograph, showing movement of male pronucleus towards animal pole. $\times 370$. (E) scheme, showing vertical section of ovum during second maturation division. $\times 370$. (F) photomicrograph, showing polar bodies of fertilized ovum in nearly vertical section. $\times 370$. (G) photomicrograph, showing fusion of male and female pronuclei in nearly vertical section. $\times 370$. *f* female pronucleus, *mp* polar view of metaphase in first maturation division, *pb* polar body, *s* sperm-nucleus or male pronucleus.

the ovum itself gives off the first and second polar bodies (Fig. 7 F). Thus, after the completion of the maturation division, the egg-pronucleus fuses with the sperm-nucleus near the animal pole (Fig. 7 G). The completion of the whole process of fertilization requires about five hours after the entrance of the spermatozoön, if the temperature of sea-water is retained at 23-25°C.

The fertilized ova inside the burrow are pushed out before long on to the surface of the sandy bottom, and an egg-mass enveloped in a jelly capsule results. Such an egg-mass is well known as the so-called *Arenicola* egg-mass, the capsule of which is supported in the sand by a jelly stalk (Fig. 8). Each egg-capsule always contains numerous fertilized ova discharged rather superficially. The shape of the egg-masses is mostly spherical, and sometimes, though rarely, ellipsoidal. The latter shape is produced only in the case of stormy conditions in the habitat. Most of the egg-masses found at Moura measure 3.5-4.5 cm. in the diameter of the capsules. But larger and smaller ones are also occasionally found. Of all the egg-masses collected during the first half of the breeding season in 1939, the largest one had a diameter of 5.2 cm. and the smallest that of 1.6 cm.

The spawning of *Arenicola cristata* is practically indicated by the formation of these egg-masses. According to the investigations during the first half of the breeding season in 1939, the formation of the egg-masses occurs nearly synchronously among all the members throughout the habitat and is repeated periodically at about four-day intervals, as shown in Table 2 and Fig. 9. And the egg-masses always appear near low water in twilight. Moreover, the ova contained in the youngest egg-mass appearing on the sandy bottom develop mostly to the two-cell or four-cell stages, and rarely to the eight-cell stage. Therefore, the actual discharge of generative products inside the burrow is presumed to occur at flood tide or at high water previous to the formation of the egg-masses.



Fig. 8 Photograph, showing egg-mass of *Arenicola cristata*.
× 2.3

TABLE 2. Periodical formation of egg-masses, investigated in stations S_1 and S_2 , during the first half of the breeding season in 1939.

Period of spawning	Date, forming egg-masses	Station S_1		Station S_2	
		No. of egg-mass	No. of casting	No. of egg-mass	No. of casting
I	Evening, 17th July	9	33	-	-
	Evening, 18th July	3	22	-	-
	Evening, 19th July	-	-	14	381
II	Morning, 22nd July	22	42	-	-
	Morning, 23rd July	-	-	6	357
	Morning, 24th July	-	-	10	372
III	Morning, 25th July	-	-	11	351
	Evening, 25th July	18	35	33	321
IV	Morning, 29th July	9	30	-	-
	Evening, 29th July	2	22	115	351
V	Evening, 2nd Aug.	9	26	99	373
VI	Morning, 7th Aug.	9	30	-	-
	Evening, 7th Aug.	5	21	125	362
VII	Evening, 11th Aug.	17	34	39	314
I'	Morning, 15th Aug.	1	26	-	-
	Evening, 15th Aug.	7	24	30	353

EXPERIMENTS ON THE DISCHARGE OF GENERATIVE PRODUCTS AND THE FERTILIZATION

The above-mentioned periodical and synchronous occurrence of the discharge of generative products and of the fertilization must be explained by ecological investigations. In this section of the paper, therefore, some elementary experiments will be described concerning the discharge of generative products and the fertilization.

EXPERIMENT I.

At first, the changes of positions of the castings at the stations S_1 and S_2 were investigated respectively twice a day during July of 1939. The result of this investigation shows that the positions of the castings were not changed in the daytime but were changed at night, and that all the castings could be divided into two groups according to their

changing grades at night, especially when the discharge of generative products was approaching: one group being mobile and the other more stationary. Moreover, by keeping the male and the female in an aquarium or by isolating them in the natural habitat, it is remarked that the former group represents the male and the latter the female. Namely, the male is very mobile during the breeding season and tunnels an intricate burrow into the sandy bottom, which becomes probably connected with that of the female at some points. In this condition, the ripe spermatozoa are discharged in the burrow. When the discharged spermatozoa are gradually scattering along the burrow and getting near the female, the discharge of ova probably results and the fertilization occurs inside the burrow of the female.

EXPERIMENT II.

The biotic influence of the male upon the sexual activity of the female was studied in this experiment, in which two different sets of the specimens were reared in the aquaria. The result of this experiment is shown in Table 3. When keeping both the male and the female in close proximity (aquaria B₁ & B₂), the formation of the egg-masses was repeated more than once and most of the ova contained in these egg-masses were fertilized. Moreover, egg-masses were produced by all the females reared there. When keeping the female alone (aquaria F₁, F₂ and F₃), the formation of the egg-masses hardly occurred at all. In aquaria F₁ and F₂, one

TABLE 3. The formation of egg-masses in rearing aquaria.

Set of experiment	Mark of aquarium	Number of specimens		Formation of egg-mass		Duration of rearing
		♂	♀	Number	Date	
Both male & female	B ₁	4	4	4	26th July	4th July -10th Aug. in 1938
				4	9th Aug.	
	B ₂	3	2	2	19th Aug.	4th Aug. -24th Sept. in 1939
				2	3rd Sept.	
				2	15th Sept.	
Female alone	F ₁	0	3	1	9th Aug.	31st July -15th Aug. in 1939
				1	11th Aug.	31st July -15th Aug. in 1939
				1	28th Sept.	4th Aug. -30th Oct. in 1939
	F ₃	0	2			

small egg-mass appeared only on 9th and on 11th August, respectively. In aquarium F₃, two females were reared for about three months, and only one formation of egg-mass was observed on 28th September. One of the two females died on 4th October, and the remaining one still retained numerous ova in its coelom at the end of the season on 30th October. On the other hand, by another experiment, in which sea-water containing discharged spermatozoa was added to the female-keeping vat, the formation of an egg-mass was observed about seven hours after the addition of this sperm-water. It is the writer's belief, therefore, that the discharge of ova is stimulated by the male.

EXPERIMENT III.

The biotic influence of the female upon the sexual activity of the male was studied in this experiment, in which the discharge of spermatozoa was observed by keeping the male in a glass vat containing sea-water alone. The result of this experiment is shown in Table 4. In the vats M₁ and M₂, a spontaneous discharge of spermatozoa took place, while, in M₃ and M₄, the discharge of spermatozoa was stimulated apparently by the addition to the vat of the female. Accordingly, the discharge of spermatozoa in the male seems to occur more readily in the proximity of the female, even when the specimens are kept under slightly abnormal condition in a vat without sand.

TABLE 4. The Discharge of spermatozoa in rearing vats.

Mark of glass vat	Number of male	Duration of rearing	Date, arising discharge of spermatozoa
M ₁	1	20th - 29th July, 1939	Early morning, 23rd July
M ₂	1	20th - 29th July, 1939	Early morning, 26th July
M ₃	1	20th - 30th July, 1939	*Early morning, 30th July
M ₄	4	16th - 19th Aug., 1939	**Early morning, 19th Aug.

*One female was added into M₃, on the evening of 29th July.

**Four females were added into M₄, on the evening of 18th Aug.

EXPERIMENT IV.

In this experiment, the effect of the temperature of the sand at a depth of about 20 cm. was studied in the station S₂ during the first half of the breeding season in 1939. The temperature was recorded by the distance-recording thermometer. As shown in Fig. 9, the temperature varies mainly according to the amount of solar radiation. In fine wea-

ther the temperature is maximum at about 6 p. m. and minimum at about 8 a. m. in the daily period. In each daily period, the difference between the maximum and the minimum temperatures is usually about 2–3°C. The discharge of the generative products is presumed to be affected by the minimum limit of the daily temperature before or in each period. When the minimum temperature was more than 23°C., the spawning occurred fully, as in the cases of periods IV, V and VI in the station S₂. At the temperature of about 20–23°C., the spawning occurred somewhat insufficiently as in the cases of periods I, II, III, VII and I', and the spawning was never observable when the minimum daily temperature was less than 20°C. The temperature increased step by step for a week before the beginning of the breeding season (Fig. 9). The temperature in the station S₁ was roughly measured and was found to be invariably higher by about 1–2°C. than that in the station S₂ during the spawning periods investigated. Accordingly, the temperature of the sand in the habitat restricts only the threshold of the spawning activity, and the temperature favourable for the spawning of the present species in this

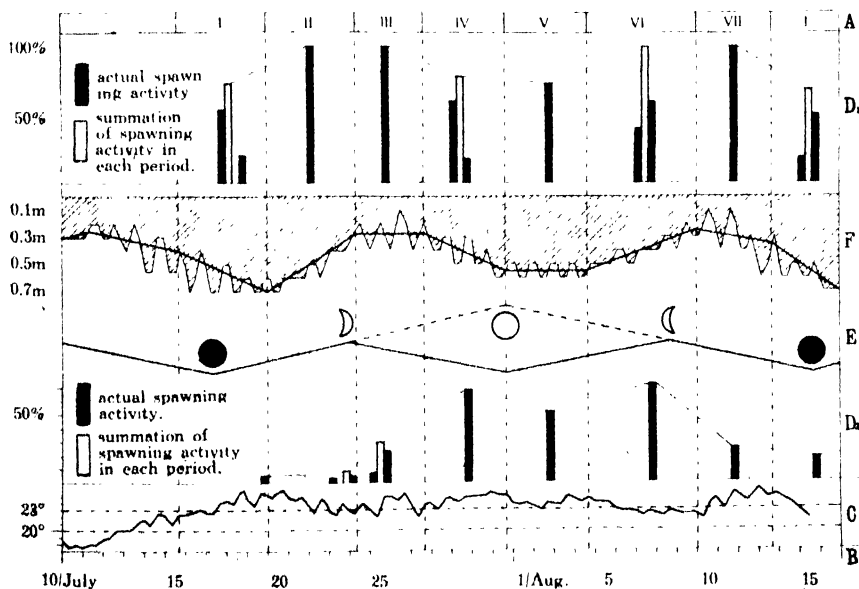


Fig. 9. Diagram, illustrating spawning periodicity and some environmental conditions during first half of breeding season in 1939. (A) spawning period, (B) date, (C) temperature-curve at depth of about 20 cm. of sandy bottom in station S₂, (D₁) periodicity of spawning activity in station S₁, (D₂) periodicity of spawning activity in station S₂, (E) lunar periodicity, (F) tidal periodicity shown by modified tide-curve of Mutu Bay.

habitat is that which is higher than 20°C. at the depth of about 20 cm. of sandy bottom.

EXPERIMENT V.

In this experiment, the effect of the tide upon the spawning periods as well as on the spawning activity was studied during the first half of the breeding season in 1939. As shown already in Table 2, the formation of egg-masses occurs at nearly regular periods. These spawning periods clearly respond to the supposed component tide at about four-day intervals, which is shown by a modified tide-curve obtained by the present writer from the tide-table of Mutu Bay²⁾. In this modified tide-curve, the abscissa represents the date and the ordinate represents the tidal ranges between the successive high and low waters (Fig. 9 F). The zigzag base of the shadowed area in the figure, therefore, indicates the variation of tidal ranges. In this variation of tidal ranges, the periods of the above-mentioned component tide (I-VII) are illustrated with seven thick base lines of the shadowed area in a lunar month. The spawning occurs usually near the centre of each tidal period during the breeding season.

As to the investigation concerning the periodicity in the spawning activity, the following formula is devised by the writer and tried in each spawning period: spawning activity = $k r \frac{E}{N}$, where k is constant depending on the egg-mass forming habit in each period, r is another constant depending on sex-ratio, E is the number of egg-masses, and N is the number of castings. The values of E and N are given in Table 2. Accordingly, the number of egg-masses produced by one female must first be investigated in each spawning period. For this investigation, males and females were isolated in the natural habitat from 10th July up to

TABLE 5. The egg-mass formation of isolated specimens in the habitat.

Set of experiment	Number of isolated specimens		Number of egg-masses			
	Male	Female	Period II	Period III	Period IV	Period V
C ₁	2	2	2	0	2	2
C ₂	4	4	2	1	4	4
C ₃	5	4	4	0	4	4

²⁾The tide-table of Mutu Bay was applied with the tide-table at Ōminato, published from the Hydrographic Department, Imperial Japanese Navy.

4th August, 1939. The result of this investigation is shown in Table 5. In every case of this investigation, the number of egg-masses produced in each period was equal to or less than the number of females isolated. Moreover, in the cases of B₁ and B₂ of Experiment II, the number of egg-masses formed in each period was always equal to the number of females isolated. From these results, the writer is under the impression that not more than one egg-mass is given invariably by one female in each spawning period: therefore, $k=1$. Secondly, the value of sex-ratio must be studied correctly as much as possible. For this purpose, two hundreds and nine specimens were collected at random during July and August in 1939, and they consisted of:—

Male.....109

Female.....100

$$\text{Therefore, } r = \frac{\text{male number} + \text{female number}}{\text{female number}} = 2$$

Thus, the spawning activity in each spawning period is approximately estimated as shown in Table 6. The spawning activity in each spawning period is variable, responding to the other component tide of fortnightly intervals, and is maximum for the period of the so-called neap-tide. This

TABLE 6. The periodical spawning activity during the first half of the breeding season in 1939.

Spawning period	I	II	III	IV	V	VI	VII	I'
Station								
S ₁	74%	100%	100%	78%	68%	100%	100%	64%
S ₂	7%	9%	30%	64%	52%	68%	25%	16%

periodicity of the spawning activity is shown typically in the station S₁ (Fig. 9 D₁). The periods I, IV, V, and I' correspond to the period of the so-called spring-tide and the spawning activity in these periods falls off to some extent. On the other hand, the periods II, III, VI, and VII correspond to the period of the so-called neap-tide and all the female are presumed to spawn in these periods. In the station S₂, the spawning activity is somewhat of a low grade and its periodicity is not evident as in the station S₁. The falling off of the spawning activity of period V in this case, however, indicates the same tidal effect in the station S₁. Another falling off of the spawning activity in periods II, III and VII may probably be caused by other environmental factors. In conclusion,

it is remarkable that the periodical spawning activity of the present species is apparently responsive to the tidal periodicity of the habitat.

REMARKS ON THE EGG-MASS FORMING HABITS

As stated already, the discharged and fertilized ova inside the burrow appear shortly after as an egg-mass on the sandy bottom. Such an egg-mass is formed by some active behaviour of the female after the shedding of ova inside the burrow. In this section of the paper, two elementary ecological investigations will be described concerning certain features of the egg-mass forming habits.

REMARK I.

The relative positions between the egg-masses and the castings were investigated in sixty instances in the station S₁. The result of this investigation shows that the relative positions between them can be divided into the following three classes; in the first class about twenty percent of the investigated egg-masses was lying at the head-depressions of the burrows, in the second class about sixty-five percent of them was lying near the head-depression of the burrows, and in the third class the remaining fifteen percent of them was lying near the castings. Therefore most of egg-masses must emerge through the opening of the head-depression of the burrow. It is supposed that a retreating movement of the spawned female inside the burrow forces the discharged ova in a contrary direction to her movement to the surface of the sandy bottom, and an egg-mass enveloped in jelly appears near the head-depression. After the formation of the egg-mass, the burrow is probably remade, as a new head-depression is found near the formed egg-mass in most cases. The first class mentioned above indicates the condition before the burrow is remade. An egg-mass was formed gently with moderate rapidity from the head-depression of a burrow in an instance observed in the glass vat. Only in a few cases, however, the egg-mass may emerge through the opening of tail-end of the burrow.

REMARK II.

The effect of solar light upon the egg-mass forming habits is considered according to Table 2, in which every formation of egg-masses occurred without fail in the twilight of morning as well as of evening. Moreover active movements of this animal were never observed in the daytime; as stated already in Experiment I. Owing to this nocturnal

activity, it is suggested that the ova discharged in the daytime remain inside the burrow till the evening, and those discharged in the night till the morning. Thus, it is presumed that solar light has a definite effect upon the active movements of this animal with relation to the formation of egg-mass.

THE EARLY DEVELOPMENT

As stated already, the youngest egg-mass appearing on the surface of the sandy bottom contains many developing zygotes at the beginning stages of cleavage. After the processes of cleavage and of gastrulation, the young larvae are developed inside this egg-capsule, and then hatch out of the capsule to grow into free swimming larvae. This early development up to the hatching stage will be described in this section of the paper.

THE CLEAVAGE.

The segmentation of this species is unequal and total from the beginning. The type of cleavage is the so-called 'spiral' one. Accordingly, a similar system to the terminology used by OKADA (1935) for *Sphaerium* (= *Musculium*) is most appropriately applied to the nomenclature of the blastomeres (Table 7 and Pl. IV, Figs. 1-19). It is a modification of WILSON's system (1892) in his 'Cell-lineage of *Nereis*'. In this observation, the specimens of the first two or three stages of cleavage were obtained as a result of artificial fertilization in the laboratory, and those of the later stages were obtained from the young egg-masses of the natural habitat. In the laboratory conditions, the temperature of the sea-water was always kept at 23-25°C.

THE FIRST CLEAVAGE (*Stage of two cells*).

The first furrow of cleavage cuts into the zygote with almost the same rapidity on all sides, separating it about vertically into two unequal parts, the smaller AB and the larger CD (Pl. IV, Fig. 1). After this cleavage, the two blastomeres, which belong to cells of the second generation if the unsegmented zygote is counted as the first, flatten together to a considerable extent, and the typical two-cell stage is produced (Pl. IV, Fig. 2). In the live specimens, the polar bodies are hardly observable, but they are easily discriminated in the sections of fixed specimens (Fig. 10). The nucleus of CD is larger than that of AB. They are lying slightly near

TABLE 7. The earliest cell-lineage of *Arenicola cristata*.

Matur- ation	1st	2nd.	3rd.	4th.	5th.	6th.	7th.	Blastula	Fate of Blastomeres																																																								
	Generation																																																																
	ca. 480 min.	30 min.	40 min.	50 min.	60 min.	80 min.		(at 23-25°C)																																																									
	uncleaved stage	2-cell.	4-cell.	8-cell.	16-cell.	(26-cell.) 32-cell.	(37-cell.) 68-cell.																																																										
Sperma- tozoan	Fertilized Ovum	AB	A	1a	1a ₁	1a ₁₁	1a ₁₁₁	1a ₁₁₁₁	Ectomeres																																																								
										1a ₂	1a ₁₂	1a ₁₁₂	1a ₁₁₂₁	1a ₁₁₂₂																																																			
															1A	2a	2a ₁	2a ₁₁	2a ₁₂	2a ₂₁	2a ₂₂																																												
																						2A	3a	3a ₁	3a ₂	4a	4A																																						
																												3A	1b ₁₁	1b ₁₁₁	1b ₁₁₁₁	1b ₁₁₁₂																																	
																																	1b ₁	1b ₁₂	1b ₂₁	1b ₂₂																													
																																					1B	2b	2b ₁	2b ₁₁	2b ₁₂	2b ₂₁	2b ₂₂																						
																																												2B	3b	3b ₁	3b ₂	4b	4B																
																																																		3B	1c ₁₁	1c ₁₁₁	1c ₁₁₁₁	1c ₁₁₁₂											
																																																							1c ₁	1c ₁₂	1c ₂₁	1c ₂₂							
																																																											1C	2c	2c ₁	2c ₁₁	2c ₁₂	2c ₂₁	2c ₂₂
3C	1d ₁₁	1d ₁₁₁	1d ₁₁₁₁	1d ₁₁₁₂																																																													
					1d ₁	1d ₁₂	1d ₂₁	1d ₂₂																																																									
									1D	2d	2d ₁	2d ₁₁	2d ₁₂	2d ₂₁	2d ₂₂																																																		
																2D	3d	3d ₁	3d ₂	4d(M)	4D																																												
																						3D	4d(M)	4D																																									

sperm- entrance	1st cl.	2nd cl.	3rd cl.	4th cl.	5th cl.	6th cleavage	7th cleavage

the animal pole. The distribution of cytoplasm is not homogeneous in each blastomere. The fine plasm is distributed round the nucleus, and the deutoplasmic large granules are deposited near the vegetal pole. At the temperature of 23–25°C., the two-cell stage was presumed to arrive about seven hours after the discharge of ova.

THE SECOND CLEAVAGE (*Stage of four cells*).

The second cleavage gives rise to cells of the third generation of blastomeres. The division arises first in CD which separates into two unequal parts, the smaller C and the larger D. Meanwhile, division also takes place in AB and two nearly equal parts A and B are separated. These two divisions often occur synchronously. The spindles of these two divisions are formed in a manner which may be called 'laetotropically' (Pl. IV, Fig. 3). Thus, the four-cell stage is produced by these two divisions, which result in three almost equal blastomeres A, B and C, and one large blastomere D (Pl. IV, Figs. 4 & 5). When the blastomeres flatten together, the polar furrows are distinct and parallel at both poles, the furrow at the vegetal pole being longer. In the sections of fixed specimens, a small intermittent cleavage cavity is often present in this stage (Fig. 11). The nucleus of D is larger than that of C, and the latter is slightly larger than those of A and B. The cytoplasmic structures of these four blastomeres are almost the same as those in the two-cell stage, and the blastomere D has the most abundant amount of the deutoplasm. In the laboratory condition, the four-cell stage was always completed about thirty minutes after the appearance of the two-cell stage.

THE THIRD CLEAVAGE (*Stage of eight cells*).

The first quartette of micromeres is formed by four divisions in this cleavage, which gives rise to cells of the fourth generation. Of these four divisions, C and D divide into the so-called macromeres 1C and 1D and the so-called micromeres 1c and 1d respectively, although D has a tendency to divide rather earlier than C. About synchronously to these two divisions, the divisions of A and B occur, and produce the so-called macromeres 1A and 1B and the so-called micromeres 1a and 1b respectively, the eight-cell stage, thus, being resulting. (Pl. IV, Figs. 6 & 7). In these four macromeres, 1D is larger than the others 1A, 1B, and 1C, each of which is about equal in size. In the first quartette of micromeres, 1d is slightly larger than the other three. The spindles of all these divisions are formed 'dexiotropically', as it may be called (Fig. 12), and

the blastomeres in the first quartette contain a far less amount of deuto-plasm than those of the vegetal half. In the laboratory condition, the eight-cell stage was generally completed about forty minutes after the formation of the four-cell stage.

THE FOURTH CLEAVAGE (*Stages of from nine to sixteen cells*).

Including the formation of the second quartette of blastomeres, the



Fig. 10



Fig. 11



Fig. 13

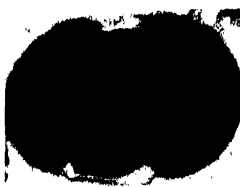


Fig. 12



Fig. 14

Fig. 10. Photomicrograph, showing median vertical section along polar axis of two-cell stage. $\times 250$.

Fig. 11. Photomicrograph, showing horizontal section perpendicular to polar axis of four-cell stage. $\times 250$.

Fig. 12. Photomicrograph, showing transverse vertical section along polar axis of four-cell stage forming eight-cell stage. $\times 250$.

Fig. 13. Photomicrograph, showing about median vertical section along polar axis of sixteen-cell stage. $\times 250$.

Fig. 14. Photomicrograph, showing sagittal vertical section along polar axis of thirty-two-cell stage. $\times 250$.

fourth cleavage gives rise to cells of the fifth generation. The synchronism among these divisions of all four quadrants begins to disappear from this stage. The division of 1D, which produces 2D and 2d, is more advanced than the others (Pl. IV, Figs. 8 & 9). This first member 2d in the second quartette is by far the largest among the blastomeres and is the so-called first somatoblast, which has been described in the cleavage of many annelids and molluscs. The divisions of 1A, 1B and 1C occur next after the formation of the first somatoblast, the smaller 2a, 2b and 2c being, thereby, separated from the larger 2A, 2B and 2C. Synchronously to the productions of 2a, 2b and 2c, the unequal divisions give rise in the first quartette of micromeres, and produce there the eight micromeres, the larger 1a₁ and the smaller 1a₂, etc., respectively. The true blastocoel begins to appear at about this stage. The deutoplasm is deposited chiefly in the four macromeres 2A, 2B, 2C and 2D (Fig. 13). Thus, the sixteen-cell stage results together with the formation of the second quartette of the blastomeres (Pl. IV, Figs. 10 & 11). The spindles of all these divisions are formed 'laetotropically', as it may be called. In the laboratory condition, the sixteen-cell stage was generally completed about fifty minutes after the formation of the eight-cell stage.

THE FIFTH CLEAVAGE (*Stages of from seventeen to thirty-two cells*).

In the fifth cleavage which gives rise to cells of the sixth generation, the first somatoblast 2d is divided unequally into the two parts, the larger 2d₁ and the smaller 2d₂, previous to the formation of the third quartette of blastomeres. Immediately after the completion of this division, the divisions of 2D and of micromeres in the first quartette occur about synchronously. The sixteen cells of the first quartette in this generation are denoted by 1a₁₁, 1a₁₂ and 1a₂₁, 1a₂₂, etc. The first member 3d in the third quartette is smaller than the so-called macromere 3D. Meanwhile, the smaller 3a, 3b and 3c are separated from the macromeres 3A, 3B and 3C respectively. About synchronously to the separation of 3a, 3b and 3c, the three other members 2a, 2b and 2c in the second quartette are also divided respectively into two equal parts; and 2a₁₁ and 2a₁₂, etc., are produced. Thus the thirty-two-cell stage is completed after the formation of the third quartette (Pl. IV, Figs. 12 & 13). The blastocoel appears clearly in the sections of the fixed specimen (Fig. 14). In the laboratory condition, the thirty-two-cell stage was generally completed about one hour after the formation of the sixteen-cell stage.

THE SIXTH CLEAVAGE (*Stages of from thirty-seven to sixty-four cells*).

Including the formation of the fourth quartette of blastomeres, the sixth cleavage gives rise to cells of the seventh generation. Strictly speaking, there is no longer any synchronism among these divisions in the four quadrants, for the more rapid rhythm of division of certain cells becomes increasingly evident. The first member 4d of the fourth quartette is separated from the macromere 4D by the division of 3D (Pl. IV, Fig. 14). About synchronously to the formation of 4d, the progenies of the first quartette, 1a₁₁, 1b₁₁, 1c₁₁ and 1d₁₁, divide respectively into two almost equal parts; and 1a₁₁₁ and 1a₁₁₂, etc., are produced. Thus, the thirty-seven-cell stage is found temporarily in the process forming the fourth quartette. After the completion of this thirty-seven-cell stage, the remaining members 4a, 4b and 4c are separated from the so-called macromeres 4A, 4B and 4C respectively. About synchronously to the separation of 4a, 4b and 4c, the remaining twelve members 1a₁₂, 1a₂₁ and 1a₂₂, etc., of the first quartette, the large progeny 2d₁ from the first somatoblast, and the four members of the third quartette divide respectively into two parts, the fifty-seven-cell stage thus appearing. (Pl. IV, Fig. 15). After the division of the large blastomere 2d₁, the three large blastomeres 2d₁₁, 2d₁₂ and 2d₂ are produced, though 2d₁₁ is slightly larger than the others. The divisions of the other members of the second quartette except 2d₁ are always delayed and occur about synchronously with the most advanced divisions of the next generation. In consequence of this delay, the sixty-four-cell stage is never actually formed. The so-called laetotropic spindle formation in all these divisions gives an impression of considerable confusion. As a matter of fact, all these divisions are no more of a spiral type, but have become irregular. In the sections of the fixed specimens, it is worth noting here that the first member 4d of the fourth quartette is the so-called mesoblast, and begins to indicate a sign of sinking into the blastocoel immediately after its formation. The other three members of the fourth quartette and the basal four macromeres contain a large amount of deutoplasm and arise at the original vegetal pole with a columnar arrangement, a sign of growing endomeres, thus, being indicated. In the laboratory condition, the sixth cleavage was generally completed about one and half hours after the formation of the thirty-two-cell stage.

THE SUBSEQUENT GENERATIONS OF CLEAVAGE (*Blastula stage*).

At this point after the seventh generation of cleavage, the main features of the blastula will be described briefly, because the division of

each blastomere becomes increasingly irregular in the subsequent generations. It is impossible to follow the lineage of a single cell beyond this stage. In the early stages of blastula, the so-called 'rosette' and 'annelidan cross' are discriminated around the original animal pole (Pl. IV, Fig. 16), and the first bilateral divisions occur in the stem-cell of the first somatoblast and in the mesoblast. After the separation of the two daughter cells $2d_2$ and $2d_{12}$, the first somatoblast $2d_{11}$ in the seventh generation may be given the designation X. This stem cell X divides

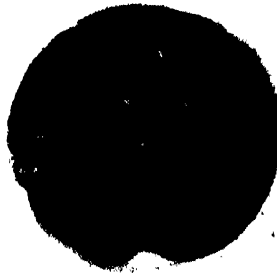


Fig. 15



Fig. 16

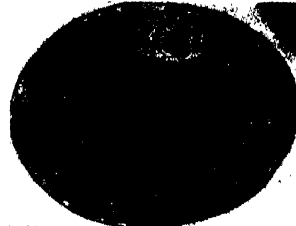


Fig. 17

Fig. 15. Photomicrograph, showing pair of first somatoblasts in early blastula stage. $\times 250$.

Fig. 16. Photomicrograph of sagittal vertical section in early blastula stage, showing invasion of mesoblast into blastocoel. $\times 250$.

Fig. 17. Photomicrograph of transverse vertical section of early blastula stage, showing first bilateral formation of mesoblastic teloblasts. $\times 250$.

equally into left and right halves, which are denoted by X_s and X_d respectively (Fig. 15). This pair of large blastomeres is to be considered as teloblastic in nature and takes part in the formation of the future somatic plate. The mesoblast $4d$ completes its action of sinking into the blastocoel at this stage (Pl. IV, Fig. 17), which may be given with the designation M, and the blastocoel itself is narrowed both by this invasion of the mesoblast and by the ingrowth of the endomeres (Fig. 16). Meanwhile,

the mesoblast **M** also divides equally into its left and right halves, which are denoted by M_s and M_d respectively. In the section of the early blastula, the pair of the large mesoblasts embedded in the blastocoel is observable as the so-called 'teloblastic cells of the mesoderm' (Fig. 17). In the laboratory condition, these developmental features of the early blastula are observed about fifteen hours after the discharge of the ova.

In the later stage of the blastula, the boundaries of the blastomeres become more indistinct. At a superficial view, therefore, the later blastula appears to be like the uncleaved egg (Pl. IV, Fig. 18). In a careful observation, however, some landmarks observed in this stage will render it possible to study the various groups of cells, and to determine approximately their fates. The progenies of the first quartette arise at the animal half, and give rise to the primary trochal regions and the intermediate girdle-cells beside the rosette and the annelidan cross. The progenies of three small members of the second quartette, and the cells of the third quartette arise at, or slightly below, the equator of the blastula; the former gives rise to the secondary trochal regions, and the latter gives rise probably to the secondary somatoblasts in each quadrant. The endomeres remain almost exactly in their original position and show an indication of increasing proliferation (Pl. IV, Fig. 19). The growth of the somatic plate becomes active from this stage, and the blastula itself begins to be elongated, the area of the originally animal pole being pushed in an opposite direction. In the laboratory condition, these developmental features of the later blastula are observed about twenty-four hours after the discharge of the ova.

THE GASTRULATION.

In the progress of development, the later blastula enters the process of gastrulation and grows to the gastrula. The archenteron is formed by a slight invagination of the endomeres and is completed mainly by an epibolic growth of the superficial area, especially by the active divisions of cells in the somatic plate. The closure of the blastopore is, therefore, explained as a consequence of the concrescence of the somatic plate in the median line. On the other hand, a rotation of the embryonic axes is completed with these processes of the gastrulation (Fig. 18). As stated above, the blastula begins to be elongated in the last stage of the cleavage. According to this elongation and the epibolic growth in the gastrulation, the originally animal pole of the zygote performs a rotation of about

ninety degrees. The rotated area around the originally animal pole eventually coincides with the future anterior side of the embryo, and the newly elongated portion of the somatic plate with the future posterior side. The area around the vegetal pole remains at about its original position and turns to the future ventral side of the embryo. Moreover, during the later stage of the gastrulation, a ciliary ring appears at the middle region of the gastrula, and the later gastrula itself may be called the trochophore. These gastrulae inside the egg-capsule are generally obtained during the second day of development.

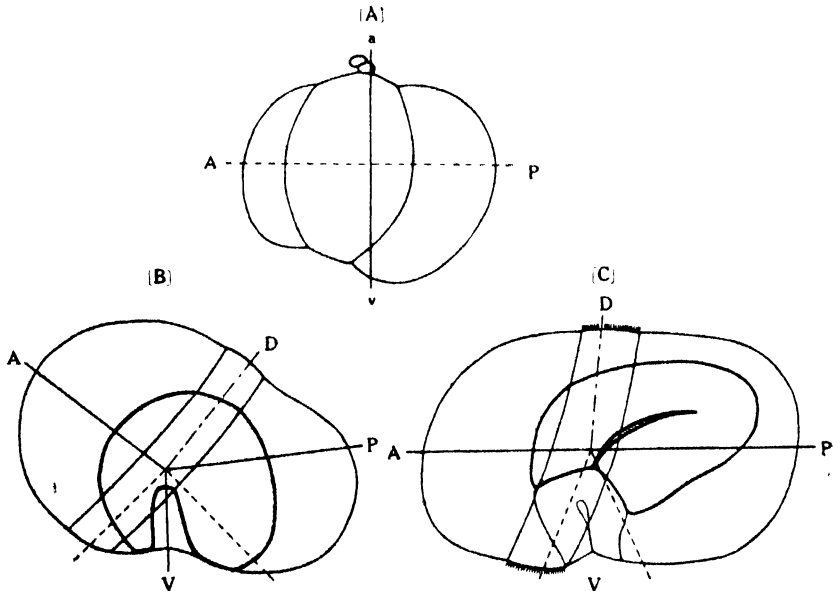


Fig. 18. Diagrams, illustrating rotation of embryonic axes in process of gastrulation. $\times 300$. (A) embryonic axes in four-cell stage, (B) same in early gastrula, (C) same in trochophore. *AP* antero-posterior axis of embryo, *av* polar axis of egg, *D* dorsal area, *V* ventral area of embryo.

THE EARLY GASTRULA.

The external form of early gastrula is an elongated pear-shape, measuring about 180μ in maximum diameter. Its round end indicates the anterior side of the gastrula and its elongated end the posterior side. In frontal views, the anterior half of the gastrula is relatively hyaline, while, the posterior half is somewhat opaque owing to the underlying endomeres and mesoblasts (Pl. IV, Fig. 20). Moreover, slight indentation occurs at the middle portion of the gastrula. In lateral views, the indenta-

tion is distinct on one side only and does not occur on the opposite side (Pl. IV, Fig. 21). This indented area indicates the margin of the blastoporal lip in this stage, though the archenteron is not observable externally. The three germ layers are discriminated in the sections of the fixed specimens (Fig. 19). The trochoblasts are shown with cubical cells and their spherical nuclei, though the cilia are not yet present in them. The posterior region of the ectoderm is constituted of the large cells which form the so-called somatic plate. The stem cells of this somatic plate are still the largest and remain at the posterior-most end of the gastrula. In the mesoderm, two kinds of cells are found in this stage: one kind

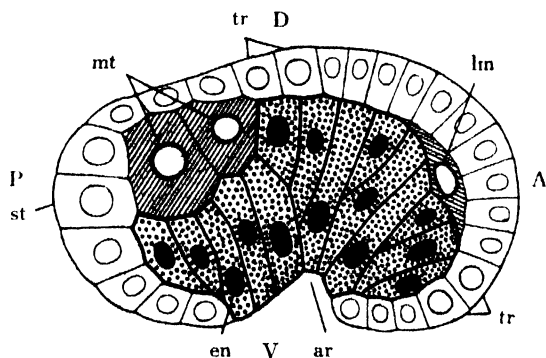


Fig. 19. Schematic illustration of parasagittal section of early gastrula, showing differentiation of three germ layers. $\times 400$. A anterior side, D dorsal side, P posterior side, V ventral side; ar archenteron, en endomere, lm larval mesoblast, mt teloblastic cell of mesoderm, st stem cell of somatic plate, tr trochoblasts.

being the four primary mesodermal cells which are produced by the divisions of the mesodermal teloblasts, and the other kind a few cells of the larval mesoblasts which lie at the anterior portion of the endomeres. The separation of these larval mesoblasts is produced independently from the primary mesoblasts. In the later blastula, they are separated either from some of the second quartette of the blastomeres or from the anterior ele-

ments of the endomeres, probably from the former. The endoderm begins to invaginate slightly, and the small archenteric cavity is found there, the blastopore being widely opened. Each endomere retains a large amount of the deutoplasmic substance. The blastocoel is completely obliterated by this process of gastrulation.

THE TROCHOPHORE (*The later gastrula*).

In the subsequent stage of gastrulation, the primitive gut is formed with the closure of the blastopore. In this later stage, the gastrula grows to be a kind of trochophore form, furnished with the rudimentary proto-troch. The external shape of the trochophore of the present species is

peculiar as shown in Pl. IV, Figs. 22 & 23. In this stage also, its anterior half remains relatively hyaline and its posterior half becomes more opaque because of yolk globules contained in the endomeres. In the well-developed trochophore, a rudimentary tuft of telotrochal cilia begins to develop near the posterior end, and the first pair of eye-spots, having reddish pigments, appear on both the latero-dorsal portions anterior to the prototroch (Pl. IV, Fig. 23). The apical tuft of cilia, which is often present on the trochophores of some other species, is not found in this case. After the complete closure of the blastopore, a sign of small ectodermal invagination is observable at the ventral portion just posterior to the prototroch.

This invagination is the rudiment of the stomodaeum. The primary mesoderm in this stage is the two mesodermal bands, formed by divisions of the teloblastic cells and occupying both ventro-lateral positions of the posterior blastocoel (Fig. 20). The larval mesoblasts are also multiplying in number by their own divisions and occupy most of the anterior portion of the blastocoelic space. The primitive gut concealed in the blastocoel is practically shown as a solid mass composed of the granular endodermal cells in the early trochophore.

In the well-developed trochophore, the rudimentary enteric lumen first appears as a slit-like space in the centre of this solid mass.

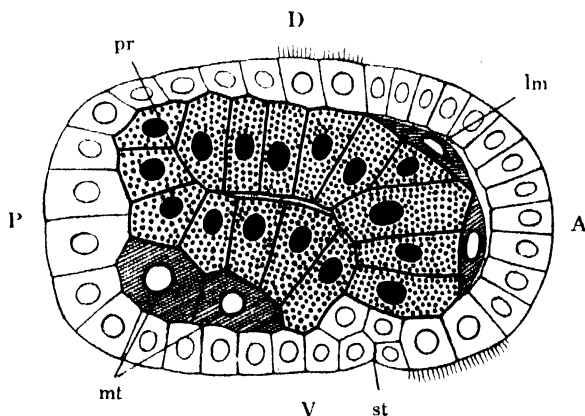


Fig. 20. Schematic illustration of trochophore in para sagittal section, showing differentiation of three germ layers. $\times 400$. A anterior side, D dorsal side, P posterior side, V ventral side; *lm* larval mesoblast, *mt* teloblastic cell of mesoderm, *pr* primitive gut, *st* stomodaeum.

THE EARLY LARVAL STAGES.

The characteristic vermicular form, known as *Arenicola* larva, is developed continuously from the trochophore stage. By the formation of the prototrochal and telotrochal ciliary rings, this larval body differentiates

into the following three regions: the head-region anterior to the prototroch, the terminal region posterior to the telotroch and the trunk region between them. In the progress of the development, the primitive metameries with pairs of the setae appear at the growing trunk region of this early larva.

On the other hand, many diatomes such as *Nitzschia longissima*, are found at the outer surface of the egg-capsule a few days after the formation of the egg-mass. The egg-capsule itself then begins to break up gradually in various places, and the larvae inside swim away through the broken spots, probably owing to the positive phototaxis of the larvae themselves, and enter the free swimming stage. This hatching of the larvae usually occurs several days after the formation of the egg-mass, when the larvae reach the stage with 3-5 pairs of the setae. Therefore, the developmental stages of the early larvae before hatching are most succinctly indicated by the number of pairs of the larval setae (Pl. V, Figs. 24-30).

THE EARLIEST LARVAE WITHOUT SETAE (Pl. V. Figs. 24 & 25).

About the beginning of the third day of development, the well-developed trochophore transforms gradually to the earliest larval form by the successive growth of the trunk region as well as the further development of the two ciliary rings. Besides these ciliary rings, a broad longitudinal band of small cilia called neurotroch begins to appear at the ventral region, and a tuft of cilia is also found at the posterior end apart from the telotroch. This earliest larva becomes contractile and is liberated from each egg-membrane inside the egg-capsule. The ectoderm of the head-region forms the two-layered epithelium by the active cell-divisions. Each trochoblast furnished with long cilia is comparatively large and quite distinct from the others. The ectoderm of the trunk region is a unicellular layer. The dorsal portion of this layer forms the thinner epithelium and is composed of flattened cells, while, the ventral and lateral portions form the thicker epithelium and are composed of cubical cells. The stomodaeum invaginates towards the anterior end of the primitive gut. The cells forming paired eye-spots increasingly deposit the pigment granules. The mesodermal bands in this stage extend and occupy the entire ventro-lateral portion of blastocoelic space on both sides. The stem-cell of each mesodermal band remains near its posterior end, and is a little larger among the mesodermal cells. The spindle-shaped mesodermal cells differentiating into the connective tissue-cells are scattered in the anterior and dorsal portions of the blastocoelic space. The anterior end of the

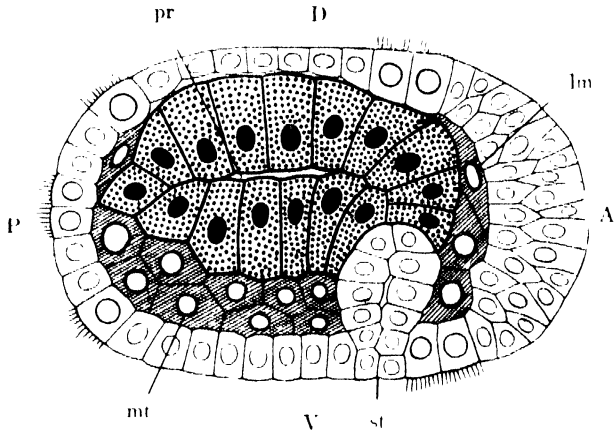


Fig. 21. Schematic illustration of earliest larva without setae in parasagittal section. $\times 400$. *A* anterior side, *D* dorsal side, *P* posterior side, *V* ventral side; *Im* larval mesoblast, *mt* teloblastic cell of mesoderm, *pr* primitive gut, *st* stomodaeum

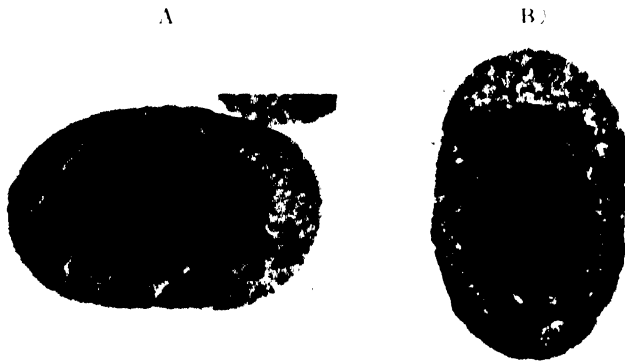


Fig. 22. Two photomicrographs of sagittal (A) and frontal (B) sections in earliest larva without setae. $\times 250$.

enteric lumen is not continuous to the stomodaeal lumen, and each endomere contains many yolk globules. (Figs. 21 & 22).

THE EARLY LARVAE WITH 1-2 PAIRS OF SETAE (Pl. V, Figs. 26, 27 & 28).

About the end of the third day of development, the trunk region of the larval body becomes segmented, and the pairs of the setae are forming in these primitive metameries. The first pair of the dorsal setae is formed on the second segment, one small spade-shaped seta appearing on each dorso-lateral side. Meanwhile, the second pair of this notopodial setae is also formed on the third segment. In this stage, the live larval body

measures about 0.2 mm. in length and 0.07 mm. in maximum breadth. These larvae swim actively inside the egg-capsule, showing a rotatory motion by means of the well-developed cilia. The eye-spots become more distinct owing to the further deposition of the reddish pigments.

The ectoderm of the head-region becomes multi-layered and vacuolated in places. Several ectodermal cells are differentiating into the nervous cells at the inner anterior end of the ectoderm. This is the rudiment of the brain. The trochoblasts constituting the prototroch bear fully-developed long cilia, and also begin to be vacuolated. The ectodermal epithelium of the trunk region is thinner in the dorsal and thicker in

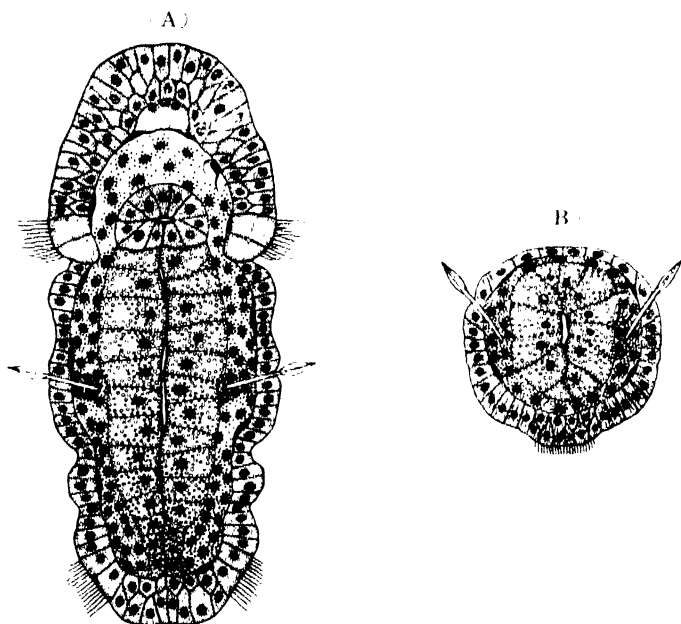


Fig. 23. Illustrations of early larva with one pair of setae, showing its inner structure. (A) frontal section, (B) same through first pair of setae. $\times 350$.

the ventral regions. The median area of the ventrally thickened epithelium is furnished with dense cilia of neurotroch. The stomodaeum elongates further and shows an S-shaped winding in the left-side view. In this stage, the trochoblasts constituting the telotroch bear also well-developed cilia. The ectoderm of the terminal region forms the two-layered epithelium, the rudimentary proctodaeum appearing there. The whole blastocoelic space is filled with mesodermal elements constituting the mesodermal bands. Some of these elements are differentiating into

chaetigerous cells. The remaining elements are loosely arranged in the outer and inner layers, no coelomic space being yet appeared. The stem cells at the posterior ends of the mesodermal bands become almost indistinct from the others. The spindle-shaped mesodermal cells differentiating into the connective tissue cells line the inner face of the ectoderm and the outer face of the endoderm. The stomodaeal lumen becomes first continuous to the enteric lumen at the anterior end, but the enteric lumen itself remains blind at the posterior end. In each endodermal cell, vacuoles are often found among many yolk globules (Fig. 23).

THE EARLY LARVAE WITH 3-5 PAIRS OF SETAE (Pl. V, Figs. 29 & 30).

After the fourth day of development, the early larva reaches the hatching stage mentioned above, and is vigorously crawling and swimming inside the egg-capsule. When hatched, the larva is about 0.3 mm. long and 0.08 mm. broad in the live condition, and is always provided with 3-5 pairs of setae (Fig. 24). In these larvae, the next following segment acquires setae rapidly. The old pairs of the notopodial setae are reenforced by the addition of new spade-shaped setae, where the old ones grow to bristles with a long, drawn-out tip. At the same time, a pair of the neuropodial setae called crotchets appears ventrally on the respective chaetigerous segment. Thus, the chaetigerous segment has two notopodial setae and a crotchet on each side, while, the last segment bears only the newly formed spade-shaped seta. In this stage, another small pair of eye-spots becomes distinct dorso-posteriorly to the first pair of eye-spots. All larval cilia are fully developed externally, including the prototroch, the telotroch, the neurotroch and the terminal tuft of fine cilia.

In the head-region, the rudimentary brain becomes more distinct, and the old pair of eye-spots becomes larger and the eyes lie at both the postero-lateral portions adjacent to the brain, showing the cup-like aggregations of the pigment granules. The newly formed eye spots are far less distinct because of the smaller deposition of pigments in this stage. In other ectodermal cells of the head-region, vacuoles appear and enlarge to produce the so-called head-vesicles. Among these vesicles, the largest one is found at the anterior side of the brain. The trochoblasts constituting the prototroch begin to indicate gradual symptoms of atrophy. The ventral portion of the ectodermal epithelium of the trunk region becomes two-layered at the level posterior to mouth, where the rudimentary ventral nervous tissue is observable. The other elements of the trunk ectoderm remain unicellular. The proctodaeum appears clearly in this stage

at the terminal ectoderm, and continues to the posterior end of the primitive gut. The trochoblasts of telotroch also begin to be vacuolated and indicate gradual symptoms of atrophy. These vacuoles enlarge in this case and grow to be the so-called tail vesicles. The differentiation of some mesodermal cells into connective tissue cells or chaetigerous cells advances more progressively, and the rudimentary coelomic space begins



Fig. 24. Photomicrographs of five-day old larvae immediately after hatching out of egg-capsule. $\times 70$.

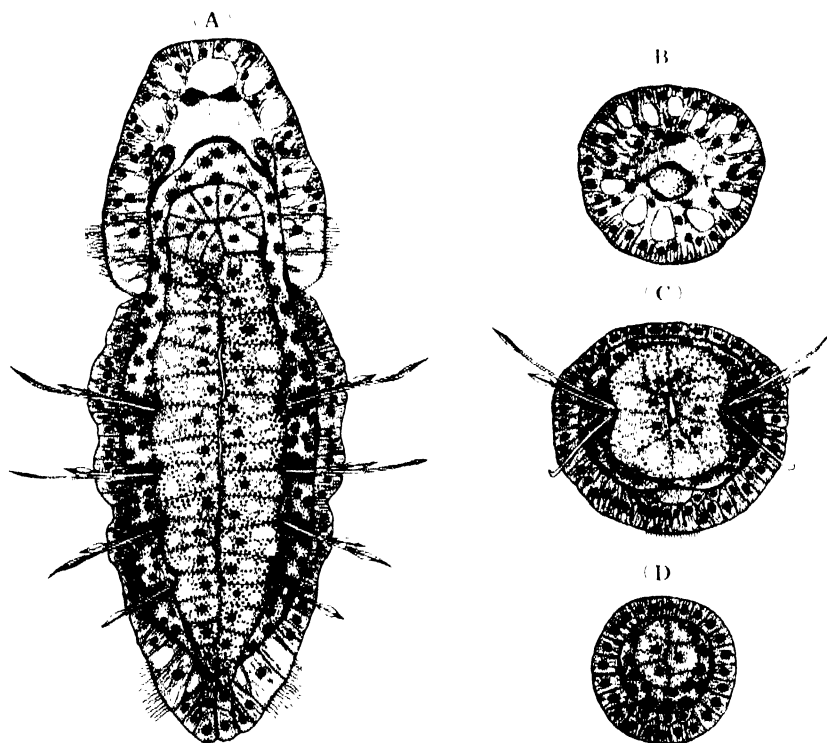


Fig. 25. Illustrations of early larva with four pairs of setae, showing its inner structure. (A) frontal section, (B) transverse section through eye-spots, (C) same through second pair of setae, (D) same through posterior end of primitive gut. $\times 350$.

to appear near the stomodaeum. Many vacuoles are found in the endodermal elements constituting the wall of the primitive gut, probably owing to the absorption of yolk globules (Fig. 25).

DISCUSSION

The preceding account concerning the embryological study of *Arenicola cristata* agrees generally with the already known processes, which have been studied by many authorities in the embryology of other polychaetes. There is, however, no agreement as to the interpretation of some of the phenomena within the scope of the present study. The relative importance of these various interpretations will be discussed as much as possible in the following paragraphs:—

THE FORMATION OF SPERMATOPHORES.

In the cases of the species belonging to Arenicolidae, the formation of the spermatophores during spermatogenesis has been reported already by ASHWORTH (1904) and DOWNING (1911). ASHWORTH gives a few figures of spermatophores in different spermatogenetic stages in the case of *Arenicola marina*. DOWNING gives an account of the spermatogenesis in the four species of Arenicolidae, viz., *Arenicola cristata*, *claparedii*, *marina*, and *grubii*. A similar formation of the spermatophores is sometimes observed in the spermatogenesis of other species belonging to various groups of animals (Turbellaria, Annelida, Gastropoda, Cephalopoda, Bryozoa, Crustacea, Amphibia, etc.). There are two different kinds of theoretical interpretations concerning the formation of the spermatophore.

Usually in such instances, the fertilization is accomplished neither internally in a strict sense, nor externally in a dispersed condition, but is accomplished by a process of the so-called pseudo-copulation or by some similar process. Thus, the spermatophores are received by the female in or near the reproductive openings, and are sometimes very elaborate affairs containing a complex mechanism arranged so as to discharge the sperm just at the time the female is depositing the eggs. By this interpretation, therefore, the cytophore forming the central protoplasmic mass of the spermatophore is nothing but an aggregation of cast cytoplasm in the process of spermioteleosis.

On the other hand, it is reported that the so-called cytophore of spermatophore takes an actual hold of the nuclei, or a few central cells become abortive during the growing process. In such instances, the cytophore or the abortive cells may be a variety of 'nurse cells' cor-

responding to the follicles of the ovum. By this interpretation, therefore, the spermatophore has a functional individuality supplying nourishment to the component spermatozoa.

DOWNING (1911) proposes an interesting interpretation concerning this individuality, that the spermatophore means the existence of the alternation of generations in the animal kingdom. He says in a section of his paper: "The conception of the alternation of generations has developed in its clear-cut simplicity among the botanists. It is that in the life history of a form there are two generations, one of which produces sexually, the other by asexual spores. It is typified in the bryophytes and most pteridophytes. In the higher plants the sexual or gametophyte generation is gradually reduced so that it is only in relatively recent times that its existence as such has been recognized in the phaenogams. The term 'alternation of generations' has been used by zoologists in a totally different sense. Two generations occur in many animals, the so-called sexual and asexual. The latter originates from a fertilized egg; the former arises by budding or a similar process from the asexual generation. There is thus an alternation of a generation that reproduces sexually with one that is never sexual, but the latter does not reproduce by asexual spores as in the case in plants. It is unfortunate that the same term is used for both processes. The asexual generation in the animal alternation is much more comparable to the sporophyte which is produced in propagation by cuttings or by runners. I am using the term 'alternation of generations' strictly as it is understood by botanists."

According to this conception, which may be theoretically correct, the spermatophore represents the gametozoon generation, and the so-called sexual individual in the significance used by many zoologists represents the sporozoon generation. In other words, the spermatogonium is a kind of asexual spore as understood by botanists and the mature spermatozoon himself is the male gamete, or the oogonium is the other kind of asexual spore and the mature ovum herself is the female gamete. Thus, the gametozoon generation is extremely reduced in the alternation of generations in the animal kingdom, similar to that in the phaenogams. The spermatophores is a clear manifestation of the gametozoon generation in the DOWNING's alternation of generations. And, the term 'alternation of generations' used by many zoologists stands for nothing but the transformation of mere life-phases within one generation.

Indeed, the spermatophore of *Arenicola cristata* is the individual that may represent a generation — the young spherical form of the spermato-

phore transforms into the disc-like mass of the ripe spermatozoa in consequence of the spermioteleosis. This transformation of the external shape of the spermatophore is not fully explained by the view that the spermatophore is a mere aggregation of the spermatozoa in order to make fertilization easy. And the DOWNING's conception of the 'alternation of generations' includes essentially the second view on the spermatophoral individuality. On the other hand, the fertilization of *Arenicola cristata* occurs externally but not in a dispersed condition. The fertilization of this animal occurs in the condition of close proximity of the male and female which acts upon their mutual biotic influences.

If the individuality of the spermatophores is re-investigated more precisely, and mechanism of the disintegration of the spermatophores is studied more fully, the theoretical significance of the formation of the spermatophores will be more clearly understood. This problem, however, will be left for later treatment.

THE SPERMATOZOA.

According to ASHWORTH's (1904) figure of the ripe spermatozoön of *Arenicola marina*, the head is clearly divisible into three, viz: — an apical body, a nucleus and a middle piece. The apical body is a flattened cap-like shape on the anterior end of the nucleus, and the middle piece is notched behind to receive the tail. In the present writer's observation, however, the head of the ripe spermatozoön of *Arenicola cristata* is club-shaped, the anterior end of the head being comparatively slender, and the three divisions of acrosome, nucleus and middle piece are much less distinct than they are in ASHWORTH's figure. These different shapes of the parts of the spermatozoön of *Arenicola* may be a specific variation in the two species, or a more possible variation due to the different spermatogenetic stages.

THE MATURATION OF OVA.

The ovarian eggs of *Arenicola cristata* are liberated from the gonad in an early stage and grow singly in the coelomic fluid. The grown ova of *Arenicola cristata* are of a spheroidal shape, somewhat flattened to the polar axis. CHILD (1900) states that the three axes in the ovum of *Arenicola cristata* are of slightly different lengths before fertilization. The maturation of the fully grown ova of *Arenicola cristata* occurs immediately after their discharge of them into the sea-water, and never occurs in the coelomic fluid. During this process, the first polar spindle

appears in a tangential position, and then rotates in a radial position after the breaking down of the germinal vesicle. Such a rotation or migration of the first polar spindle has been known in the case of some other species. COE (1899) observes the rotation of the first polar spindle in the case of *Cerebratulus*. MEAD (1898) and LILLIE (1906) observe the migration of the first polar spindle in the case of *Chaetopterus*. And, LILLIE states that the migration of the polar spindle may be accompanied by the cytoplasmic stream caused by the rupture of the germinal vesicle, and by the polarization of the egg-structure independent of the fertilization, and that the cause of the spindle rotation is not so significant as it has been exaggeratedly interpreted. MEAD has given a very careful account of the origin of the asters of the maturation divisions in *Chaetopterus*. According to his account, a large number of asters arise around the ruptured germinal vesicle; two of these, which he calls the primary asters, become large and create the spindle of the maturation divisions. Indeed in the writer's observation, the polarization of the egg-structures seems to be caused by the rupture of the germinal vesicle independent of the fertilization, and the rotation of the spindle does not always seem a mere insignificant consequence of the polarization. During this rotation, one or more extra-asters appear in the residual substance of the germinal vesicle.

The formation of these extra-asters in the egg of *Arenicola cristata* is certainly normal: these extra-asters are of some interest in comparison with MEAD's observation of the multiple asters in the *Chaetopterus* egg. The writer is under the impression that this extra-aster formation in the first maturation division may possibly be associated with the rotation of the spindle, because the extra-asters vanish invariably with completion of the spindle rotation. It is safe to maintain, however, that the problem concerning this rotation does not positively fall within the scope of the present paper.

THE PERIODICITY IN REPRODUCTION.

Periodicity in reproduction has been reported by many writers in the case of various species of animals and plants. As shown in Table 8, these animals are found belonging to notably dispersed groups --- the seven phylla Coelenterata, Platyhelminthes, Annelida, Mollusca, Arthropoda, Echinodermata and Vertebrata. Such a periodicity in the reproduction of animals is interpreted, in most cases, as the periodicity responding to the lunar phases. But, as a matter of fact, the actual occurrence of

TABLE 8. A list of animals in which periodical reproduction has been found by many writers.

Taxonomical position	Species name	Author
Coelenterata		
Hydrozoa	<i>Obelia geniculata</i>	ELMHIRST (1926)
Actinozoa	<i>Pocillipora bulbosa</i>	MARSHALL & STEPHENSON (1933)
	<i>Fungia actiniformis</i>	ABE (1937)
Platyhelminthes		
Turbellaria	<i>Convoluta roscoffensis</i>	GAMBLE & KEEBIE (1903)
Annelida		
Polychaeta	<i>Eunice viridis</i>	WHITMEE (1875), FRIEDLAENDER (1898), KRÄMER (1899)
Errantia	<i>Eunice fucata</i>	MAYER (1902)
	<i>Lysidice oele</i>	HORST (1905)
	<i>Odontosyllis enopla</i>	GALLOWAY & WELCH (1911)
	<i>Ceratocephale osawara</i>	IZUKA (1903)
	<i>Nereis japonica</i>	IZUKA (1908)
	<i>Nereis limbata</i>	LILLIE & JUST (1913)
	<i>Platynereis dumerili</i>	HEMPELMANN (1911)
	<i>Platynereis megalops</i>	JUST (1914)
	<i>Platynereis</i> sp.	AIYAR & PANIKKAR (1937)
	<i>Eulalia punctifera</i>	FAGE & LEGENDRE (1926)
Polychaeta		
Tubicola	<i>Amphitrite ornata</i>	SCOTT (1909)
	<i>Polyphthalmus pictus</i>	FAGE & LEGENDRE (1926)
	<i>Arenicola cristata</i>	OKADA
Mollusca		
Amphineura	<i>Chiton tuberculatus</i>	CROZIER (1922)
	<i>Chaetopleura apiculata</i>	GRAVE (1922)
	<i>Acanthozostera gemmata</i>	STEPHENSON (1934)
Pelecypoda	<i>Ostrea edulis</i>	ORTON (1926)
	<i>Cumingia tellinoides</i>	GRAVE (1927)
	<i>Pecten opercularis</i>	AMIRTHALINGAM (1928)
Gastropoda	<i>Siphonaria atra</i>	ABE (1939)
	<i>Siphonaria siphon</i>	ABE (1939)
	<i>Siphonaria japonica</i>	ABE (1939)
	<i>Melarhaphe scabra</i>	ABE (1939)
Echinodermata		
Echinoidea	<i>Toxopneustes variegatus</i>	TUNNETT (1910)
	<i>Centrechinus setosus</i>	FOX (1924)
Arthropoda		
Insecta	<i>Clunio pacificus</i>	OKA (1930)
	<i>Clunio marinus</i>	CASPER (1939)
Vertebrata		
Pisces	<i>Leuresthes tenuis</i>	THOMPSON (1911), CLARK (1925)
	<i>Anstrocobitis attenuatus</i>	WHITLEY (1935)
Mammalia	<i>Homo sapiens</i>	ARRHENIUS (1898)

reproduction is observed as taking place at all times of the moon's phases in different localities. For example, HEMPELMANN (1911) reports, in the case of *Platynereis dumerilii* at Naples, the actual swarming at the time of the first and third quarters of the moon, JUST (1914) reports, in the case of *Platynereis megalops* at Woods Hole, the maximum of the swarming activity at the periods of the full and new moon, and AIYAR and PANIKKAR (1937) state that *Platynereis* sp. in Madras Harbour swarms at the periods of the new moon in March, July and September. On the other hand, the maximum reproduction at the time of the full or new moon is reported in every case investigated at Woods Hole, viz., in the cases of *Nereis* (LILLIE & JUST 1913), *Platynereis* (JUST 1914), *Amphitrite* (SCOTT 1909), *Chaetopleura* (GRAVE 1922) and *Cumingia* (GRAVE 1927). It is presumed, therefore, that the similar periods of the animal reproduction are not proper to the taxonomical characteristics, but appear to be a common sequence in a definite locality, though of course they are different in details. In the case of *Arenicola cristata* near the Asamusi Marine Biological Station, the periodical discharge of the generative products is also observable at about four-day intervals during the breeding season, and the two maxima of the spawning activity in a lunar month are found at the periods of both the first and third quarters of the moon.

No adequate general cause has been yet decided upon concerning the so-called lunar periodicity in reproduction in many animals as well as in plants. The two chief variable influences involved in the lunar periodicity are the light of the moon and the tide. The assumption supported by HEMPELMANN and others, is that in the life-history of animals their maturing depends mainly on the effect of the light of the moon, and only partly on the lunar tidal variations and the rhythmical alternations of conditions of nutrition. AMIRTHALINGAM (1928) denies, however, experimentally, the positive effect of the light of the moon upon the maturing of *Pecten*, and his assumption is, in short, that physiological rhythm which has originated mainly from the tidal periodicity brings on the periodical reproduction of the animals. FRIEDLAENDER (1898) and GRAVE (1922) held that the variable water-pressure produced by the flood and ebb tides causes directly the periodical reproduction. MAYER (1902) is, however, experimentally against FRIEDLAENDER's opinion. And also, HEMPELMANN, LILLIE and JUST are of opinion that the tidal ranges at Naples or Woods Hole are too small to affect the natural swarming stimulus directly. Nevertheless, the rhythmical changes of the cumulative environmental effect caused by tidal periodicity are always very complicated: tidal

periodicity is connected with all the rhythmical alternations of the physical and chemical conditions of the sea-water. Tidal force results mainly from the attractive force of the moon, and partly from that of the sun, and is also related to meteorological factors to a certain degree. The actual periods of the tide vary in different localities. And the tidal periodicity in a definite locality is a summation of various component tides—seven kinds of component tides (about half-day, one-day, four-day, half-month, one-month, half-year and one-year periods) can be detected in the tidal periodicity near the Asamusi Marine Biological Station. GAMBLE and KEEBLE (1903) state, in the case of *Convoluta roscoffensis*, that the diurnal and fortnightly variations in the size of the colonies of this creature are tidal and lunar, and that the colonies increase to a maximum during spring-tides and decrease to a minimum during neap-tides and that this is due to the periodicity of reproduction. MARSHALL and STEPHENSON (1933) divided a lunar month into eight periods, and investigated the delivery of the planula in each period throughout ten months, in the case of *Pocillipora bulbosa* in the Law Isles. According to the result of the investigation the delivery of the planula is observable at the periods of the new moon during the summer, and of the full moon during the winter. It is suggested, therefore, that the delivery of the planula in *Pocillipora* depends more closely on the component tide of about half-year periodicity than on the phases of the moon. AMIRTHALINGAM (1928) reports, also, lunar periodicity in reproduction of *Pecten opercularis* near Plymouth. And he concludes that the lunar periodicity is due to the cumulative effect of the tide, the food and the direct light of the moon. Furthermore, he says:—“it is suggested that in the animal there is a physiological rhythm that causes the development of the gonad to coincide with the full moon of each lunar month”.

As mentioned above, seven spawning periods are found in a lunar month throughout the breeding season in the case of *Arenicola cristata* near Asamusi, and the maxima of the spawning activity are observed in the periods of the neap-tides in the first and third quarters of the moon. These spawning periods coincide well with a series of component tides of about four-day periodicity, and the maxima of the activity with other series of component tides of fortnightly periodicity. Such a close response of the reproduction to the tidal periodicity is very remarkable, and suggests that the physiological rhythm has arisen on account of some relation of the life-history to the tidal periodicity, though the production of the gametes in the coelom appears to be continuous practically during the

maturing season. The writer is of opinion that the periodicity in the spawning of *Arenicola cristata* is probably a physiologically acquired character affected apparently by tidal periodicity, and that the tidal periodicity is responsible for the cumulative effect of various environmental factors.

As a matter of course, the temperature, the food and the solar light affect the spawning activity, but they do not directly affect the periodicity of the spawning activity, at least in the case of *Arenicola cristata*. ORRÖN's opinion (1926) that the temperature regulates the duration of the breeding of some animals, is applicable fundamentally to the breeding habits of *Arenicola*. The extraordinary drop in the spawning activity at the beginning and the end of curve D₂ in Fig. 9, is probably due to the fall of the temperature of the sandy bottom.

The food supply has also little tidal periodical effect upon the feeding of *Arenicola*. Accordingly, the nature of the sand, where the animals burrow, effects essentially and consecutively their growth, as shown in Table 1. Thus, the writer is under the impression that the spawning activity of low grade and of the spoiled periodicity at the station S₂ in comparison with that at the station S₁, is greatly due to the meagre condition of nutrition at the former station. As to the effect of light upon the maturing of *Arenicola*, no positive investigation was taken in the present study. It is certain, however, that the formation of egg-masses in twilight is caused by the effect of the solar light upon the egg-mass forming activity of *Arenicola*. This problem is beyond the scope of the present study and will be left for later treatment.

THE EARLY LARVAL FORM.

In spite of the abundance of writings dealing with the early development of the polychaetes, particularly with the earliest cell-lineage in the cleavage and gastrulation, there are only a few which give complete information concerning the developmental changes of the early larval stages. The cell-lineage of *Arenicola cristata* is fully described by CHILD (1900), nothing being written about its larval stages. ASHWORTH (1904) gives also only brief and generally unsatisfactory information about the early development of *Arenicola clapedii*. Thus, the present study is the first occasion on which a complete series of information about the early development of *Arenicola cristata* is given in detail up to the free swimming stage. The cleavage of the present specimens follows quite similar lines to those described by CHILD and ASHWORTH. And the precise

account on cleavage given by CHILD tallies very well with the present observation. There remains no room for discussion of the comparison of the cleavage of *Arenicola* with that of other polychaetes.

On the other hand, the embryological knowledge about the trochophores and the early larvae of the polychaetes is so far unsatisfactory as a whole. The most complete information about the larval stages of the polychaetes, belonging to the genera *Nereis*, *Nephtys*, *Pectinaria*, *Audouinia* and *Branchiomma*, have recently been given by D. P. WILSON (1932, '36a, '36b & '36c). Some interesting comparisons between the early larva of *Arenicola* and those of neighbouring families reported by WILSON, can be pointed out : —

The trochophore and the early larva of *Arenicola cristata* are provided with relatively poor ciliary organs, and are yolky without spacious blastocoel. According to the information given by WILSON, there are various kinds of specializations for the pelagic life in the polychaete larvae. The larva of *Nereis pelagica*, highly specialized for the pelagic life, is provided with a prototroch, an akrotrach and three paratrochs, and also has long bristles. The trochophore of *Nephtys homergi* is provided with five kinds of ciliary organs, viz., an apical tuft of cilia, three rings of well-developed prototroch, a curious long tuft of several cilia posterior to the prototroch, a pair of small curved lines of cilia rather ventral in front of the prototroch, and a broad neurotroch at the ventral area. In the larval forms of *Pectinaria koreni*, the ciliation is very complicated ; a conspicuous apical tuft of cilia, a prototroch, a tuft of long cilia edging the mouth, a narrow neurotroch and a telotrochal tuft of cilia are provisionally observed in its trochophore. In the larval forms of *Audouinia tentaculata* also, the ciliation is rather complex. A broad prototroch, an apical tuft of long and short fine cilia, a weakly developed telotroch, and a few sensory cilia at the posterior end are observed in its trochophore, while, in the more developed larva, the apical tuft of long cilia disappears, their place being taken by several fine cilia regarded as a degenerating akrotrachal cilia, and a weakly developed gastrotroch in addition to a broad neurotroch appears also at its ventral area. The larva of *Branchiomma vesiculosum* is provided with a broad prototroch, a broad neurotroch, but no telotroch.

In these ciliary features, and also in general shape, *Arenicola* larva shows a transitory form between *Audouinia* and *Branchiomma* larvae, with a greater resemblance to the latter form. The anterior two paratrochs of *Nereis* larva are presumably homologous to the feebly developed gastrotroch and the neurotroch of *Audouinia* larva, while, the larva of *Arenicola*

possesses no gastrotroch. The posterior-most paratroch of *Nereis* larva is presumably homologous to the telotroch of both *Audouinia* and *Arenicola* larvae, while, the larva of *Branchiomma* does not possess this structure. The absence of ciliation in the head region is common to the larvae of both *Arenicola* and *Branchiomma*. The accumulation of yolk and other relatively similar organizations in both larvae are responsible for the poor specialization for their pelagic life. The further comparison of larval structures with those of a number of other similarly constructed larvae gives an accurate demonstration of the systematic relationships among the polychaetes. Precise information available for attempting this comparison is still more imperfect and generally unsatisfactory as matters stand.

SUMMARY

1) In the present paper, the gametogenesis, the breeding habits and the early development are described embryologically in the case of *Arenicola cristata* STIMPSON near the Asamusi Marine Biological Station.

2) The gonads are closely associated with six pairs of nephridia in both the male and the female.

3) The spermatogonia in mass-formation called spermatophores are liberated from the testes into the coelomic fluid. Each spherical spermatophore undergoes the spermatogenesis without disintegrating the mass-formation and transforms into the disc-like mass of ripe spermatozoa called the sperm-disc in the coelom.

4) The sperm-discs are discharged outside the body through six pairs of nephridial tubules. After the discharge, each sperm-disc begins to disintegrate and many spermatozoa swim away separately.

5) The spermatozoön consists of a club-shaped head and a long attenuate tail. The head measures about 4μ in length. The tail is nearly ten times as long as the head.

6) The young oöcytes are liberated from the ovary into the coelomic fluid at a early oögenetic stage and grow to the primary oöcytes there. Each fully-grown oöcyte is spheroidal; the two diameters in frontal view are about equal and measure 150μ , while, the third diameter in side view measures 120μ . This third axis coincides with the future polar axis of the mature egg. The germinal vesicle with a germinal spot lies a little eccentrically towards the animal pole. The deutoplasm surrounded by a distinct vitelline membrane nearly homolecithal and is opaque with brownish-red pigments contained.

7) The fully-grown oöcytes are discharged outside the body through six pairs of nephridial tubules. After the discharge, the germinal vesicle in each ovum breaks down and the karyokinetic figure of maturation appears near the animal pole.

8) The first spindle of the maturation division is formed in a tangential position before the spermatozoön enters, and remains to be the metaphase after its rotation to a radial position. The extra-asters are normally observable during the rotation of the first polar spindle.

9) The spawning of this species near Asamusi begins about the middle of July and continues with a characteristic rhythm of activity to about the middle of September. The maturing of the generative products begins a little previously to and covers the spawning season.

10) During the breeding season, the external discrimination between the male and the female becomes distinct by the colouring of numerous generative products in the coelom.

11) The actual discharge of the generative products and the fertilization occur inside the burrow of the sandy bottom with the male and the female in close proximity. Several hours after the discharge of generative products, the egg-mass enveloped with jelly capsule appears on the sandy bottom.

12) The egg-capsule is usually spherical but rarely ellipsoidal with a stalk embedded in the sand, measuring mostly 4 cm. or more in diameter, and contains numerous fertilized eggs distributed rather superficially.

13) The formation of egg-masses is observed about synchronously and periodically among all the members throughout the habitat.

14) The synchronism in the spawning is caused mainly by some biotic influences between the male and the female.

15) The periods of the spawning with about four-day intervals coincide well with component tides of about four-day periodicity, and the two maxima of the spawning activity in a lunar month depend on the so-called neap-tides of fortnightly periodicity, the dates of which can, in confirmation of this, be obtained from the tide-table of Mutu Bay.

16) The periodicity in the spawning activity of this species presumably originates from the physiological rhythm due to cumulative environmental effect, responding to the tidal periodicity.

17) The spawning activity is affected essentially by the temperature and the conditions of the nutrition. The temperature favourable for the spawning of this species near Asamusi is higher than 20°C. at the depth of about 20 cm. of sandy bottom.

18) The appearance of the egg-mass on the sandy bottom is observed always in the twilight. This egg-mass forming habit is caused probably by the nocturnal activity of this species inside the burrow.

19) The young larval forms are developed inside the egg-capsule, after the processes of cleavage and of gastrulation.

20) The table of the earliest cell-lineage is given on the spiral cleavage of this species.

21) The archenteron is formed mainly by the epibolic growth of the somatic cells. The mesoderm originates mainly from the teloblastic mesoblast.

22) The rotation of the embryonic axes occurs during the gastrulation.

23) The morphological features are described precisely in the trochophore stage and in the early larval stage.

24) The early larva with 3-5 pairs of setae hatches out of the egg-capsule to grow to be the free-swimming larva several days after the formation of the egg-mass.

25) The discussion on the spermatophore, the spermatozoon, the maturation of ovum, the periodicity in reproduction and the young larval form is reported.

REFERENCES

- ABE, N. 1940. The Homing, Spawning and other Habits of a Lumpet, *Siphonaria japonica* DONOVAN. Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. 15.
- AMIRTHALINGAM, C. 1928. On the Lunar Periodicity in Reproduction of *Pecten opercularis* near Plymouth. J. Mar. Biol. Assoc. U. K., Vol. 15 (N. S.).
- ASHWORTH, J. H. 1904. *Arenicola*. L.M.B.C. Memoirs. London.
- CASPER, H. 1939. Über Lunar-Periodizität bei marinen Chironomiden. Zool. Anz., Sup.-Bd. 12.
- CHILD, C. M. 1900. The Early Development of *Arenicola* and *Sternaspis*. Arch. Ent.-mech. Org., Bd. 9.
- COE, W. R. 1899. The Maturation and Fertilization of the Egg of *Cerebratulus*. Zool. Jahrb., Bd. 12, Abt. Anat. u. Ontog.
- CROZIER, W. J. 1922. An Observation on the Cluster-formation of the Sperm of *Chiton*. Amer. Nat., Vol. 56.
- DOWNING, E. R. 1911. The Formation of the Spermatophore in *Arenicola*, and a Theory of the Alternation of Generation in Animals. J. Morph., Vol. 22.
- FOX, H. M. 1922. Lunar Periodicity in Reproduction. Proc. Roy. Soc. London, Ser. B., Vol. 95.
- GAMBLE, G. W. & F. KEEBLE 1903. The Bionomics of *Convoluta roscoffensis*, with Special Reference to its Green Cells. Quart. Jour. Micro. Sci., Vol. 47.
- GRAVE, B. H. 1922. An Analysis of the Spawning Habits and Spawning Stimuli of *Chaetopleura apiculata*. Biol. Bull., Vol. 42.

- GRAVE, B. H. 1927. An Analysis of the Spawning Habits and Spawning Stimuli of *Cumingia tellinoides*. Biol. Bull., Vol. 52.
- IZUKA, A. 1903. Observations on the Japanese Palolo. J. Coll. Sci. Univ. Tokyo, Vol. 17.
- IZUKA, A. 1908. On the Breeding Habit and Development of *Nereis japonica* n. sp. Annot. Zool. Jap., Vol. 6.
- JUST, E. E. 1914. Breeding Habit of Heteronereis Form of *Platynereis megalops* at Woods Hole, Mass. Biol. Bull., Vol. 27.
- LILLIE, F. R. 1906. Observations and Experiments concerning the Elementary Phenomena of Embryonic Development in *Chaetopterus*. J. Exp. Zool., Vol. 3.
- LILLIE, F. R. & E. E. JUST 1913. Breeding Habit of Heteronereis Form of *Nereis limbata* at Woods Hole, Mass. Biol. Bull., Vol. 24.
- MARSHALL, S. M. & T. A. STEPHENSON 1933. The Breeding of Reef Animals. I. The Corals. G. B. Reef. Exp. (1928-29), Sci. Rep., Vol. 3.
- MEAD, A. D. 1898. The Origin and Behaviour of the Centrosomes in Annelida Egg. J. Morph., Vol. 14.
- OKA, H. 1930. Morphologie und Oekologie von *Clunio pacificus* EDWARDS. Zool. Jahrb., Bd. 59, Abt. Syst.
- OKADA, K. 1935. Some Notes on *Musculium heteroden* (PILSBRY), a Freshwater Bivalve. III. Fertilization and Segmentation. Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. 10.
- OKUDA, S. 1938. Notes on the Spawning Habit of *Arenicola Claparedii* LEVINSEN. Annot. Zool. Jap., Vol. 17.
- OKUDA, S. 1939. Lunar Periodicity in Reproduction. Bot. & Zool., Vol. 7 (in Japanese).
- ORTON, J. H. 1920. Sea-temperature, Breeding and Distribution in Marine Animals. J. Mar. Biol. Assoc. U. K., Vol. 12.
- ORTON, J. H. 1926. On Lunar Periodicity in Spawning of Normally Grown Falmouth Oyster (*O. edulis*) in 1925, with a Comparison of the Spawning Capacity of of Normally Grown and Dumpy Oysters. J. Mar. Biol. Assoc. U. K., Vol. 14.
- SCOTT, J. W. 1909. Egg-laying Habits of *Amphitrite ornata* VERILL. Biol. Bull., Vol. 17.
- STEPHENSON, A. 1934. The Breeding of Reef Animals. II. Invertebrates other than Corals. G. B. Reef Exp., (1928-29) Sci. Rep., Vol. 3.
- TAKAHASHI, K. 1934. Contribution to the Study of Japanese Arenicola. I. Notes on the Habits and Distribution of *Arenicola* in Japan. Sci. Rep. Tokyo Bunrika Daigaku, Sec. B, Vol. 1.
- WILSON, E. B. 1892. The Cell-lineage of *Nereis*. J. Morph., Vol. 6.
- WILSON, D. P. 1932. The Development of *Nereis pelagica* LINNAEUS. J. Mar. Biol. Assoc. U. K., Vol. 18 (N. S.).
- WILSON, D. P. 1936. The Development of the Sabellid *Branchiomma vesiculosum*. Quart Jour. Micro. Sci., Vol. 78.
- WILSON, D. P. 1936. Notes on the Early Stages of Two Polychaetes, *Nephtys hombergi* LAMARCK and *Pectinaria isoreni* MALGREN. J. Mar. Biol. Assoc. U. K., Vol. 21 (N. S.).
- WILSON, D. P. 1936. The Development of *Audouinia tentaculata* (MONTAGU). J. Mar. Biol. Assoc. U. K., Vol. 20 (N. S.).

EXPLANATION OF PLATE IV

- Fig. 1. Two-cell stage immediately after first cleavage, viewed from animal pole. $\times 200$.
- Fig. 2. Typical two-cell stage, viewed from animal pole. $\times 200$.
- Fig. 3. Two-cell stage forming second cleavage spindle, viewed from animal pole. $\times 200$.
- Fig. 4. Four-cell stage, viewed from animal pole. $\times 200$.
- Fig. 5. Four-cell stage, viewed from right side. $\times 200$.
- Fig. 6. Eight-cell stage, viewed from animal pole. $\times 200$.
- Fig. 7. Eight-cell stage, viewed from right side. $\times 200$.
- Fig. 8. Nine-cell stage forming fourth cleavage spindle. $\times 200$.
- Fig. 9. Nine-cell stage, viewed from vegetal pole. $\times 200$.
- Fig. 10. Sixteen-cell stage, viewed from animal pole. $\times 200$.
- Fig. 11. Sixteen-cell stage, viewed from vegetal pole. $\times 200$.
- Fig. 12. Thirty-two-cell stage, viewed from animal pole. $\times 200$.
- Fig. 13. Thirty-two-cell stage, viewed from vegetal pole. $\times 200$.
- Fig. 14. About thirty-seven-cell stage viewed from vegetal pole, showing first separation of mesoblast. $\times 200$.
- Fig. 15. About fifty-seven-cell stage, viewed from animal pole. $\times 200$.
- Fig. 16. Early blastula viewed from animal pole, showing first bilateral division of first somatoblast. $\times 200$.
- Fig. 17. Early blastula viewed from vegetal pole, showing sinking of mesoblast into blastocoel. $\times 200$.
- Fig. 18. Blastula viewed from animal pole. $\times 200$.
- Fig. 19. Blastula viewed from vegetal pole. $\times 200$.
- Fig. 20. Later blastula viewed from animal pole. $\times 200$.
- Fig. 21. Later blastula viewed from right side. $\times 200$.
- Fig. 22. Early gastrula viewed from dorsal side. $\times 200$.
- Fig. 23. Later gastrula viewed from dorsal side. $\times 200$.

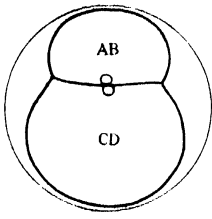


Fig. 1

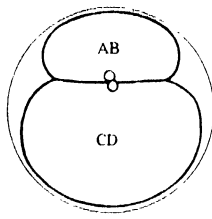


Fig. 2

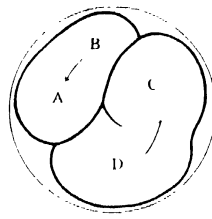


Fig. 3

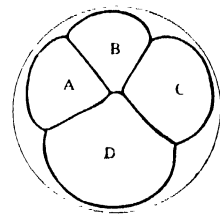


Fig. 4

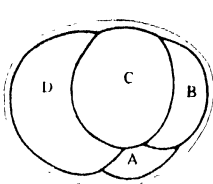


Fig. 5

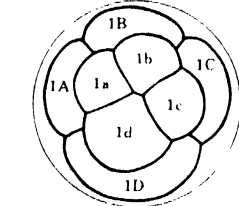


Fig. 6

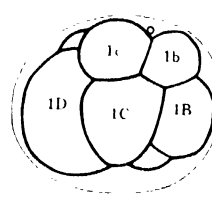


Fig. 7

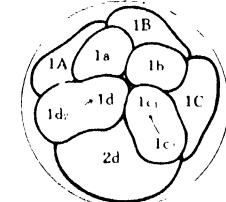


Fig. 8

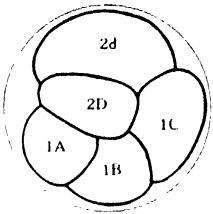


Fig. 9

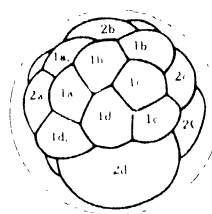


Fig. 10

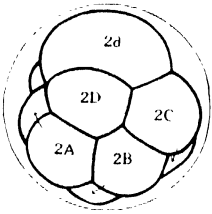


Fig. 11

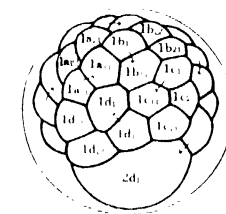


Fig. 12

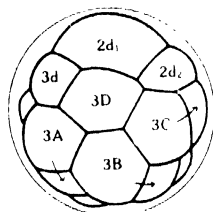


Fig. 13

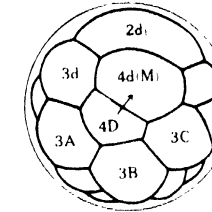


Fig. 14

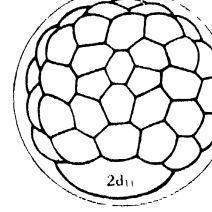


Fig. 15

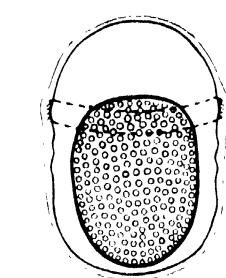


Fig. 22

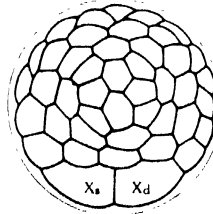


Fig. 16

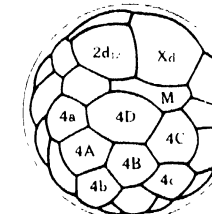


Fig. 17

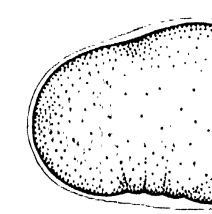


Fig. 21

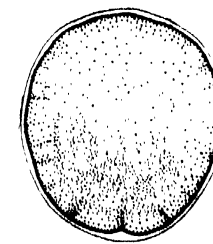


Fig. 18

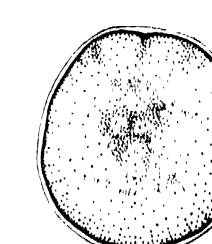


Fig. 19

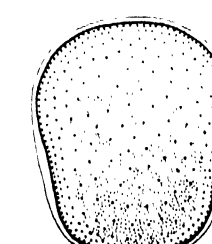


Fig. 20

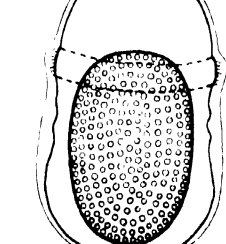


Fig. 23

EXPLANATION OF PLATE V

- Fig. 24. Earliest larva without setae, viewed from dorsal side. $\times 200$.
Fig. 25. Earliest larva without setae, viewed from right side. $\times 200$.
Fig. 26. Early larva with one pair of setae, viewed from dorsal side. $\times 200$.
Fig. 27. Early larva with one pair of setae, viewed from right side. $\times 200$.
Fig. 28. Early larva with two pairs of setae, viewed from dorsal side. $\times 200$.
Fig. 29. Early larva with three pairs of setae, viewed from right side. $\times 200$.
Fig. 30. Early larva with five pairs of setae, viewed from right side. $\times 200$.

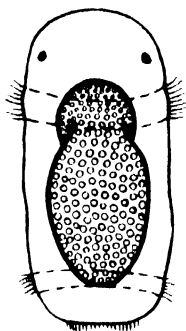


Fig. 24

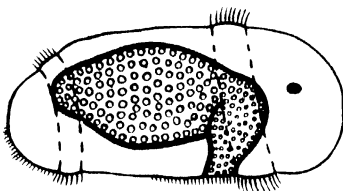


Fig. 25

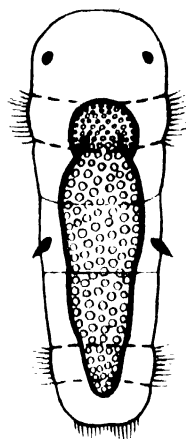


Fig. 26

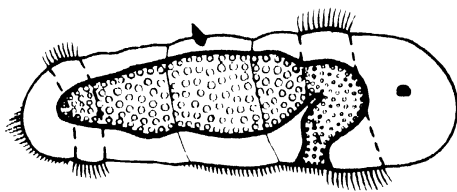


Fig. 27

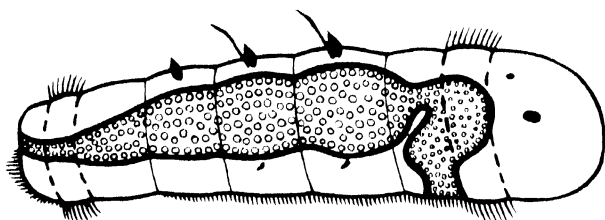


Fig. 29

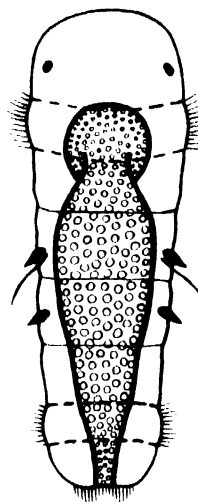


Fig. 28

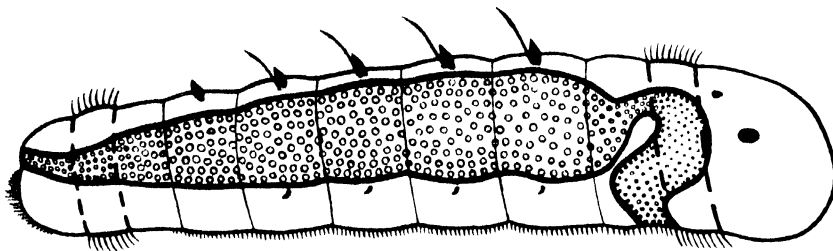


Fig. 30

EXPLANATION OF PLATE VI

Fig. 31. Ventral view of fully mature male. Natural size

Fig. 32. Dorsal view of fully mature female. Natural size

(These two figures were painted by Mr. K. AOYAMA.

Fig. 31

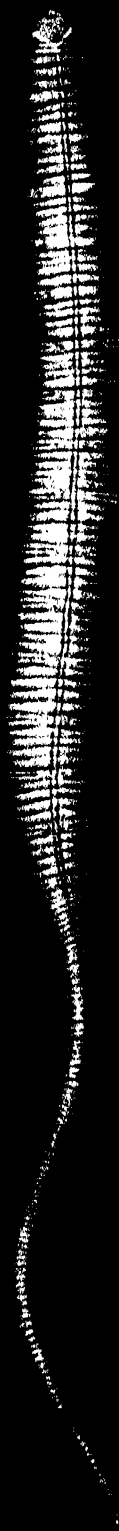


Fig. 32



EARTHWORMS OF KOREA. II.

By

SHINJIRÔ KOBAYASHI

Keijô Second Higher Common School

(With 2 Text-figures)

(Received November 8, 1940)

The paper now presented is the second report on the earthworms of Korea and it deals with the species belonging to the family Lumbricidae which were collected during 1933-36 from various parts of the Korean Peninsula. Many of these specimens were kindly presented to the present writer by friends, among them being Messrs. YOSIKI TAMURA, RYÛHON SAITÔ, TUNEITI KAMITA and TUKIJI SATÔ.

In carrying out the present study about two thousand specimens were examined, and they were found to be referable to the following ten forms: *Eisenia foetida*; *E. nordenskiöldi* f. *typica*; *Allolobophora caliginosa* f. *typica* and f. *trapezoides*; *All. japonica* f. *typica*, f. *gigantica* and f. *minuta*; *Bimastus parvus*; *B. beddardi*; *B. tenuis*. Of these ten forms, only the three forms of *All. japonica* are endemic; most of the specimens being of the two forms *E. nordenskiöldi typica* and *All. caliginosa trapezoides*. In order to make a more detailed report on the distribution of the family in Korea, further study is desirable. But, the following facts may at least be noticed viz. *E. nordenskiöldi typica* which is common in Siberia and North Manchoukuo is also plentiful in the northern highlands of the peninsula, while *All. caliginosa trapezoides* which has a world-wide distribution is the most predominant except in these regions where the former form is found.

In this place the writer wishes to express his hearty thanks to Prof. Dr. SANJI HÔZAWA for continual guidance rendered him during the course of his study. Grateful thanks must be extended to Prof. Dr. SHINKISHI HATAI, Prof. Dr. HARUJIRÔ KOBAYASHI, and to Prof. Dr. TAMEZÔ MORI for their kindness and encouragement given throughout the work. Further, the writer must pay his sincere thanks to the gentlemen already mentioned for their great kindness in supplying the materials.

1. *Eisenia foetida* (SAV.) 1826

- 1924 *Allolobophora foetida*, SASAKI, Sci. Rep. Tôhoku Imp. Univ., Biol., I, 1, pp. 89-90.
 1935 *Eisenia foetida*, ČERNOSVITOV, Monogr. tschechosl. Lumb., pp. 34-36.
 1935 *Eisenia foetida*, KOBAYASHI, Zool. Mag., 47, p. 130.
 1936 *Eisenia foetida*, KABURAKI and MISAKA, Zool. & Botany in Nikkô, p. 514.
 1936 *Eisenia foetida*, NOMURA and KOBAYASHI, Zool. Mag., 48, pp. 885-893.
 1937 *Eisenia foetida*, ČERNOSVITOV, Rec. Ind. Mus., XXXIX, 2, p. 107.
 1937 *Eisenia foetida*, TÉTRY, Bull. Mus. Hist. Nat., IX, p. 143.
 1938 *Eisenia foetida*, TÉTRY, *ibid.*, X, p. 74.
 1938 *Eisenia foetida*, KOBAYASHI, Annot. Zool. Japon., XVII, 3-4, p. 415.
 1938 *Eisenia foetida*, KOBAYASHI, Jour. Chosen Nat. Hist. Soc., XXIV, pp. 6-18.
 1940 *Eisenia foetida*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XV, 3, p. 289.

Localities in Korea: North Kankyô-dô: Seisin, Kyôjô; South Kankyô-dô: Hokusei, Eikô, Tokugen, Genzan; N. Heian-dô: Sozan; S. Heian-dô: Junsen, Tinnampo; Kôkai-dô: Shariin, Kaishû, Hakusen; Keiki-dô: Keijô, Kaijô; Kôgen-dô: Sempo, Kinkwa, Chûmonsinsin, Tetugen; S. Chûsei-dô: Kwaizan; N. Keishô-dô: Kinsen; S. Keishô-dô: Kan-an, Kiho; N. Zenra-dô: Zenshû, Riri, Kinzan, Kunsan; S. Zenra-dô: Shôteiri, Mokpo.

2. *Eisenia nordenskiöldi* (EISEN) f. *typica* 1872

- 1910 *Helodrilus (Eisenia) nordenskiöldi typica*, MICHAELSEN, Ann. Mus. Acad. Imp. Sci. St.-Petersburg, XV, pp. 17-18.
 1924 *Eisenia nordenskiöldi typica*, SVETLOV, Bull. Inst. Res. Biol. Perm., 2, p. 322.
 1929 *Eisenia nordenskiöldi typica*, MICHAELSEN, Ann. Mus. Zool. Acad. Sci. URSS., XXX, p. 329.
 1940 *Eisenia nordenskiöldi typica*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XV, 3, p. 282.

Localities in Korea: N. Kankyô-dô: Kamisampô, Kwainci, Kyôjô, Gyodaisin, Yûki, Mt. Kwambô (2000-2500 meters in altitude, within the roots of *Rheum coreanum* NAKAI, obtained by Mr. RYÛHON SAITÔ), Sanchô, Nôjidô, Mt. Mutohô (near the summit of Mt. Hakutô, within the roots of *Rhododendron chrysanthum* PALLA); S. Kankyô-dô: Hutempo (by Mr. YOSHIKI TAMURA), Keizantin, Hôzan, Huseinrei (by Mr. TUKIJI SATÔ), Simbujô-Hôtairi ('wondering on a mountainous path'); S. Heian-dô: Yôtoku; N. Heian-dô: Myôkôsan, Kôshô, Singishû, Kyûjô, Kisen.

Description: Length 44-120 mm, greatest diameter 2.5-6.8 mm, number of segments 101-176 (measured and counted in 827 complete specimens). Myôkôsan-, Kwainci-, Kisen- and Yôtoku-specimens are smaller than the rest; in these specimens, the body size varies within the range of 50×2.5 mm- 78×4.8 mm, usually being about 50×3.0 mm. In the remainder of the specimens, it varies within the range of 48×4.4 mm-

- GRAVE, B. H. 1927. An Analysis of the Spawning Habits and Spawning Stimuli of *Cumingia tellinoides*. Biol. Bull., Vol. 52.
- IZUKA, A. 1903. Observations on the Japanese Palolo. J. Coll. Sci. Univ. Tokyo, Vol. 17.
- IZUKA, A. 1908. On the Breeding Habit and Development of *Nereis japonica* n. sp. Annot. Zool. Jap., Vol. 6.
- JUST, E. E. 1914. Breeding Habit of Heteronereis Form of *Platynereis megalops* at Woods Hole, Mass. Biol. Bull., Vol. 27.
- LILLIE, F. R. 1906. Observations and Experiments concerning the Elementary Phenomena of Embryonic Development in *Chaetopterus*. J. Exp. Zool., Vol. 3.
- LILLIE, F. R. & E. E. JUST 1913. Breeding Habit of Heteronereis Form of *Nereis limbata* at Woods Hole, Mass. Biol. Bull., Vol. 24.
- MARSHALL, S. M. & T. A. STEPHENSON 1933. The Breeding of Reef Animals. I. The Corals. G. B. Reef. Exp. (1928-29), Sci. Rep., Vol. 3.
- MEAD, A. D. 1898. The Origin and Behaviour of the Centrosomes in Annelida Egg. J. Morph., Vol. 14.
- OKA, H. 1930. Morphologie und Oekologie von *Clunio pacificus* EDWARDS. Zool. Jahrb., Bd. 59, Abt. Syst.
- OKADA, K. 1935. Some Notes on *Musculum heterodon* (PILSBRY), a Freshwater Bivalve. III. Fertilization and Segmentation. Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. 10.
- OKUDA, S. 1938. Notes on the Spawning Habit of *Arenicola Claparedii* LEVINSSEN. Annot. Zool. Jap., Vol. 17.
- OKUDA, S. 1939. Lunar Periodicity in Reproduction. Bot. & Zool., Vol. 7 (in Japanese).
- ORTON, J. H. 1920. Sea-temperature, Breeding and Distribution in Marine Animals. J. Mar. Biol. Assoc. U. K., Vol. 12.
- ORTON, J. H. 1926. On Lunar Periodicity in Spawning of Normally Grown Falmouth Oyster (*O. edulis*) in 1925, with a Comparison of the Spawning Capacity of of Normally Grown and Dumpy Oysters. J. Mar. Biol. Assoc. U. K., Vol. 14.
- SCOTT, J. W. 1909. Egg-laying Habits of *Amphitrite ornata* VERILL. Biol. Bull., Vol. 17.
- STEPHENSON, A. 1934. The Breeding of Reef Animals. II. Invertebrates other than Corals. G. B. Reef Exp., (1928-29) Sci. Rep., Vol. 3.
- TAKAHASHI, K. 1934. Contribution to the Study of Japanese Arenicola. I. Notes on the Habits and Distribution of *Arenicola* in Japan. Sci. Rep. Tokyo Bunrika Dai-gaku, Sec. B, Vol. 1.
- WILSON, E. B. 1892. The Cell-lineage of *Nereis*. J. Morph., Vol. 6.
- WILSON, D. P. 1932. The Development of *Nereis pelagica* LINNAEUS. J. Mar. Biol. Assoc. U. K., Vol. 18 (N. S).
- WILSON, D. P. 1936. The Development of the Sabellid *Branchiomma vesiculosum*. Quart Jour. Micro. Sci., Vol. 78.
- WILSON, D. P. 1936. Notes on the Early Stages of Two Polychaetes, *Nephtys hombergi* LAMARCK and *Pectinaria isoreni* MALGREN. J. Mar. Biol. Assoc. U. K., Vol. 21 (N. S).
- WILSON, D. P. 1936. The Development of *Audouinia tentaculata* (MONTAGU). J. Mar. Biol. Assoc. U. K., Vol. 20 (N. S).

EXPLANATION OF PLATE IV

- Fig. 1. Two-cell stage immediately after first cleavage, viewed from animal pole. $\times 200$.
- Fig. 2. Typical two-cell stage, viewed from animal pole. $\times 200$.
- Fig. 3. Two-cell stage forming second cleavage spindle, viewed from animal pole. $\times 200$.
- Fig. 4. Four-cell stage, viewed from animal pole. $\times 200$.
- Fig. 5. Four-cell stage, viewed from right side. $\times 200$.
- Fig. 6. Eight-cell stage, viewed from animal pole. $\times 200$.
- Fig. 7. Eight-cell stage, viewed from right side. $\times 200$.
- Fig. 8. Nine-cell stage forming fourth cleavage spindle. $\times 200$.
- Fig. 9. Nine-cell stage, viewed from vegetal pole. $\times 200$.
- Fig. 10. Sixteen-cell stage, viewed from animal pole. $\times 200$.
- Fig. 11. Sixteen-cell stage, viewed from vegetal pole. $\times 200$.
- Fig. 12. Thirty-two-cell stage, viewed from animal pole. $\times 200$.
- Fig. 13. Thirty-two-cell stage, viewed from vegetal pole. $\times 200$.
- Fig. 14. About thirty-seven-cell stage viewed from vegetal pole, showing first separation of mesoblast. $\times 200$.
- Fig. 15. About fifty-seven-cell stage, viewed from animal pole. $\times 200$.
- Fig. 16. Early blastula viewed from animal pole, showing first bilateral division of first somatoblast. $\times 200$.
- Fig. 17. Early blastula viewed from vegetal pole, showing sinking of mesoblast into blastocoel. $\times 200$.
- Fig. 18. Blastula viewed from animal pole. $\times 200$.
- Fig. 19. Blastula viewed from vegetal pole. $\times 200$.
- Fig. 20. Later blastula viewed from animal pole. $\times 200$.
- Fig. 21. Later blastula viewed from right side. $\times 200$.
- Fig. 22. Early gastrula viewed from dorsal side. $\times 200$.
- Fig. 23. Later gastrula viewed from dorsal side. $\times 200$.

120 × 6.8 mm, usually being about 60 × 4.5–5.0 mm. Thus, the body-size appears to vary greatly according to the locality. Colour is also variable; in most of the specimens dorsally it is violet-red (some lighter than others), rarely dusty red or dark greenish red. Generally, the worms found in the mountainous regions are darker in colour.

Setae a and b of XVI, and of XXVII–XXXV (or some of them) may be planted on whitish tumescences. These setae are nearly straight and are grooved at the distal about 1/4–1/3, and are about 0.53 mm long and 29 μ thick in the thickest middle portion.

Clitellum saddle-shaped, usually in XXVI or XXVII–XXXIII; rarely, it begins in XXV or in 1/nXXVI, and ends in 1/nXXXIII, XXXII or invades a little, but dorsally only, on XXXIV. Pubertatis tubercles usually form distinct grooves with ridges, in XXIX–XXXI; in only two cases they were found in XXVIII–XXX and in 1/2XXVIII–1/2XXXI.

Remarks: About one thousand specimens of *E. nordenskiöldi* were examined. All these were identified with f. *typica* not even a single specimen representing f. *manshurica* being included. The forma *manshurica* has been recorded from Manchoukuo and has the clitellum in XXVI–XXXIV and the pubertatis tubercles in XXIX–XXXII.

In the northern part of the peninsula this Siberian Lumbricid earthworm is plentiful as it is also in Central and North Manchoukuo, but in North-Central Korea it is found only in the region of the highlands such as Yôtôku, Kisen and Myôkôsan. The distribution of this form in Korea is illustrated in Fig. 1.

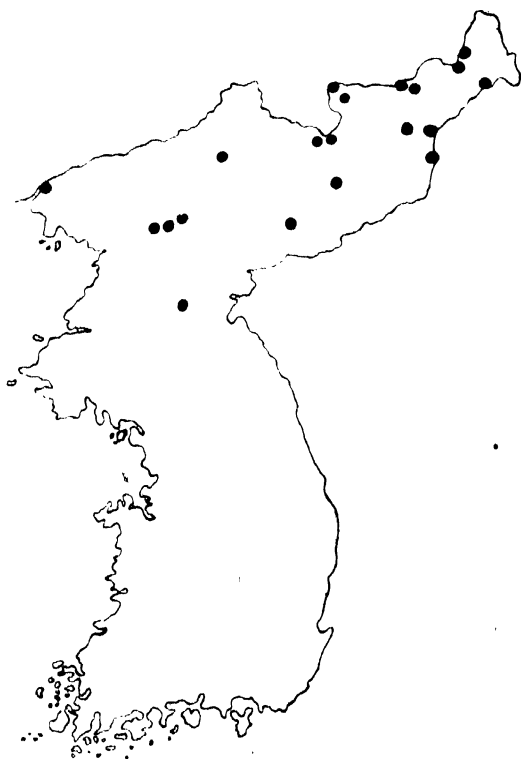


Fig. 1. The distribution of *E. nordenskiöldi typica* in Korea.

3. *Allolobophora caliginosa* (SAV.) f. *typica* 1826.

- 1924 *All. caliginosa typica*, SVETLOV, Bull. Inst. Rech. Biol. Perm, II, p. 323.
 1926 *All. caliginosa typica*, PICKFORD, Nat. Hist. Wicken Fen, III, p. 228, figs. 4, A & B.
 1931 *All. caliginosa typica*, MICHAELSEN, Zool. Jahrb. Syst., p. 537.
 1934 *All. caliginosa typica*, SCIACCHITANO, Fauna Parco Naz. Gran Paradiso, III, p. 4.
 1934 *All. caliginosa typica*, ČERNOSVITOV, Monogr. tschechosl. Lumb., pp. 52-53, fig. 35. (Literature: see this paper).
 1937 *All. caliginosa typica*, ČERNOSVITOV, Mitt. Königl. Naturwiss. Inst., X, p. 85.
 1937 *All. caliginosa typica*, TÊTRY, Bull. Mus. Hist. Nat., IX, pp. 146-148.
 1940 *All. caliginosa typica*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XV, 3, p. 295.

Localities in Korea: Tetuzan, N. Heian-dô, 23 clitellate and semi-cl. specimens, VIII/'36; ? Hakusen, Kôkai-dô, 1 semi-cl. and 2 a clitellate sps., III/'34; Keijô, 2 cl. sps., 4/V/'33, by Mr. TUNEITI KAMITA; Ryûjin, Keiki-dô, 11 cl. sps., 10/VI/'34.

Description: Length 67-160 mm, greatest diameter 3.8-6 mm, number of segments 125-160. Colour is variable; dorsally whitish-grey, pinkish or dark brown; clitellum flesh, grey or brownish-grey. Setae a and b of IX-XI are planted on oval whitish papillae; in most of the specimens, those of XXX and XXXII (or sometimes those of XXIX-XXXIII) are found on irregularly elevated papillae. Clitellum in XXVI, XXVII-XXXIV; rarely it begins in 1/XXXV. Pubertatis tubercles are of two pairs of papillae in XXXI and XXXIII; although the two papillae (puberty walls) on each side do not form a glassy, longitudinal groove as in the case of f. *trapezoides*, they are often indistinctly separated from each other.

4. *Allolobophora caliginosa* f. *trapezoides* (ANT. DUG.) 1828

- 1926 *All. caliginosa trapezoides*, PICKFORD, Nat. Hist. Wicken Fen, III, p. 228, fig. 4, c.
 1931 *All. caliginosa trapezoides*, CHEN, Contr. Biol. Lab. Sci. Soc. China, Zool. Ser., VII, 3, pp. 168-169.
 1935 *All. caliginosa trapezoides*, CHEN, *ibid.*, IX, 6, pp. 216-217, figs. 11 & 12.
 1934 *All. caliginosa trapezoides*, SCIACCHITANO, Arch. Zool. Italiano, XX, p. 14.
 1935 *All. caliginosa trapezoides*, ČERNOSVITOV, Monogr. tschechosl. Lumb., p. 53 (Literature: see this paper).
 1935 *All. caliginosa trapezoides*, ČERNOSVITOV, Mem. Soc. Zool. Tscécosl. Prague, III, p. 4.
 1935 *All. caliginosa trapezoides*, KOBAYASHI, Zool. Mag., 47, p. 130.
 1936 *All. caliginosa trapezoides*, NOMURA and KOBAYASHI, *ibid.*, 48, pp. 885-893.
 1937 *All. caliginosa trapezoides*, ČERNOSVITOV, Mitt. Königl. Naturwiss. Inst., X, p. 85.
 1937 *All. caliginosa trapezoides*, GAVRILOV, Zool. Anz., 118, p. 146.
 1938 *All. caliginosa trapezoides*, KOBAYASHI, Annot. Zool. Japon., XVII, 3-4, p. 414.

1938 *All. caliginosa trapezoides*, KOBAYASHI, Jour. Chôsen Nat. Hist. Soc., XXIV, p. 6.

1940 *All. caliginosa trapezoides*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XV, p. 296.

Localities in Korea: N. Kankyô-dô: Jôsin; S. Kankyô-dô: Hokusei, Eikô, Genzan, Tokugen, Kôgen, Reidôri, Ampen, Kankô; N. Heian-dô: Teishû, Tetuzan, Sharenkwan; S. Heian-dô: Junsen, Chôrin; Kôkai-dô: Hakusen, Angaku, En-an, Ginsen, Shariin, Sainei; Kôgen-dô: Kotei, Kôjô, Tetugen, Chûmonsins, Bunmaku, Sinkôzan, Kôryô, Genshû; Keiki-dô: Monsan, Anjô, Suigen, Risen, Kôka, Rishû, Sin-i, Rensen, Heitaku, Gumpojô, Heiten, Keijô; S. Chûsei-dô: Gasan, Taiden, Hankyô, Moshu, Reizan, Zuizan, Chôtiin, Seikwan, Tôsin; N. Chûsei-dô: Kwaizan, Teisen; N. Zenra-dô: Kunsan, Kintei, Riri, Kinzan, Nangen; S. Zenra-dô: Mokpo, Junten, Reisui, Shôteiri, Kainan, Tintô, Tanyô; N. Keishô-dô: Bunkei, Kinsen; S. Keishô-dô: Kiho, Kyôsen, Sinshû, Kan-an.

Remarks: In spite of the fact that forma *typica* are rarely found, *f. trapezoides* are very plentiful in the cultivated land in most parts of the peninsula, except in the northern highlands where *Eisenia nordenskiöldi f. typica* is predominant. About 50% of all the Lumbricid specimens examined in this study were of the present form.

5. *Allolobophora japonica* MICHAELSEN *f. typica* 1891

(Fig. 2 a & b)

1891 *All. japonica* (part), MICHAELSEN, Abh. Ver. Hamburg, XI, 2, p. 6.

1892 *All. japonica* (part-Enosima), MICHAELSEN, Arch. Naturg., 58, pp. 22-24

1893 *All. japonica* (part?) ROSA, Mem. Acc. Torino, II, 43, pp. 424 & 449.

1895 *All. japonica* (part), BEDDARD, Monogr. Oligochaeta, p. 718.

1900 *All. japonica* (part), MICHAELSEN, Tierreich, p. 481.

1903 *All. japonica* (part?), MICHAELSEN, Geogr. Verbr. Oligochaeta, p. 138.

1910 *All. japonica* (part?), MICHAELSEN, Ann. Mus. Zool. Acad. Imp. Sci. St.-Petersburg, VI, p. 62.

1934 *All. japonica typica*, ÔISHI, Zool. Mag., 46, p. 134.

1936 *Helodrilus* (*All.*) *japonica*, KABURAKI & MISAKA, Zool. & Bot. Nikkô, p. 514.

1936 *All. japonica*?, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XI, 1, p. 183.

1936 *All. japonica*, NOMURA & KOBAYASHI, Zool. Mag., 48, p. 885.

1938 *All. japonica*, KOBAYASHI, Annot. Zool. Japon., XVII, 3-4, p. 414.

1938 *All. japonica*, KOBAYASHI, Journ. Chôsen Nat. Hist. Soc., 24, p. 6.

Localities in Korea and materials: Kôryô, Keiki-dô, 2 cl. and 1 juv. sps., VII/34; Ryûjin, Keiki-dô, 1 cl. sp., 10/VI/34.

Description: Body-size 42×3.5 mm and 68×4.3 mm in Kôryô-specimens, and 57×3.6 mm in a Ryûjin-specimen; number of segments 123-133. Colour in formalin uniformly whitish-grey. Prostomium, epilobous ca. 1/2. First dorsal pore in 4/5.

Setae closely paired, moderate in size; those on the hinder part are always enlarged. Setal distance $aa > bc$, $ab > cd$ and $dd > \frac{1}{2}u$; setal ratio taken on a segment immediately posterior to the clitellum is as follows: $aa : ab : bc : cd : dd : \frac{1}{2}u = 28 : 3 : 14 : 2 : 93 : 80$. Ventral setae are larger than the lateral; the setal size examined on a segment immediately posterior to the clitellum is as follows: ventral ones are 0.39 mm long and 36μ thick in the thickest nodular portion, and the lateral ones are 0.31 mm long and 25μ thick. The ventral and lateral setae are different in shape from each other; the former are nearly straight and the latter are slightly curved at the proximal end. (Fig. 2, b). In a Ryûjin-specimen setae a and b of XXV (left side only) are planted on an oval papilla, but these are similar in shape and in size to the rest of the setae.

Clitellum saddle-shaped, in 1/nXXIII or XXIV-XXXI. Pubertatis tubercles are of two pairs of papillae on XXVII and XXIX; each papilla is triangular in shape and placed just dorsal to the ventral setae. (Fig. 2, a).

Male pores minute, on a very slight elevation on XV, about midway between b and c; the elevation may be sometimes very inconspicuous. Spermathecal pores, two pairs in 9/10 and 10/11, in line with the lateral setae.

Seminal vesicles, four pairs in IX-XII; the anterior two pairs are much smaller than the posterior two; those in X are a little smaller than those in IX. Spermathecae are very small, ovoidal, each with an inconspicuous stalk.

6. *Allolobophora japonica* f. *gigantica* ÔISHI 1934

- 1891 *All. japonica* (part), MICHAELSEN, Abh. Ver. Hamburg, XI, 2, p. 6.
 1892 *All. japonica* (part-Hakodate), MICHAELSEN, Arch. Naturg., 58, pp. 22-24.
 1893 *All. japonica* (part?), ROSA, Mem. Acc. Torino, II, 43, pp. 424 & 449.
 1895 *All. japonica* (part), BEDDARD, Monogr. Oligochaeta, p. 718.
 1900 *All. japonica* (part), MICHAELSEN, Tierreich, p. 481.
 1903 *All. japonica* (part?), MICHAELSEN, Geogr. Verbr. Oligochaeta, p. 138.
 1910 *All. japonica* (part?), MICHAELSEN, Ann. Mus. Zool. Acad. Imp. Sci. St.-Petersburg, XV, p. 62.
 1934 *All. japonica gigantea*, ÔISHI, Zool. Mag., 46, p. 134.
 1938 *All. japonica gigantea*, KOBAYASHI, ibid., 50, p. 520.

Locality in Korea and material: Kwaizan, N. Chûsei-dô, 3 cl. and 4 acl. and juv. sps., VIII/35.

Description: Unfortunately, in all the specimens the posterior parts are incomplete; but the greatest diameter is 3.5-4.2 mm. Colour in formalin, uniformly darkish-red except the clitellum which is fleshy. Setae

are larger than those of the other two forms; on a segment immediately posterior to the clitellum the ventral setae are about 0.54 mm long and 47μ thick in the thickest nodular portion. Pubertatis tubercles are triangular but somewhat roundish. (Fig. 2, c). The other characteristics are almost similar to those of f. *typica*.

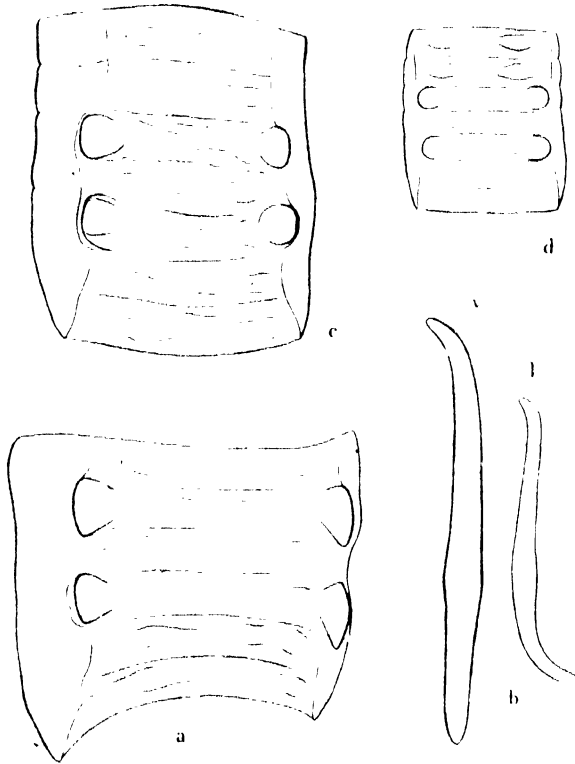


Fig. 2. *Allolobophora japonica*. a, f. *typica*, ventral view of the clitellar region; b, f. *typica*, v-ventral one, l-lateral one, \times ca. 147; c, f. *gigantica*, ventral view of the clitellar region; d, f. *minuta*, ventral view of the clitellar region; a, c & d, \times ca. 12.

7. *Allolobophora japonica* f. *minuta* ÔISHI 1934

(Fig. 2, d).

1934 *All. japonica minuta*, ÔISHI, Zool. Mag., 46, p. 134.

Localities in Korea and materials: Ryûjin, Keiki-dô, 2 clitellate and 1 aclitellate specimens, 10/VI/'34; Kôryô, Keiki-dô, 3 cl. and 1 semicl. sps., VII/'34.

Description: Body-size 24×2 mm– 31×2.5 mm in Kôryô-specimens,

and 32×2.3 mm and 40×2.8 mm in Ryûjin-specimens. Number of segments 85-110. Colour in formalin, uniformly whitish-grey. Setae are smaller than those of *f. typica*; the ventral ones are about 0.29 mm long and 24μ thick in the thickest nodular portion (those examined being on a segment immediately posterior to the clitellum). In all the specimens, setae a and b of XXV and XXVI are planted on oval papillae, but they are similar in size and in shape to the rest of the setae. Pubertatis tubercles are roundish in shape and are less distinctly demarcated than in the case of *f. typica*. (Fig. 2, d). The other characteristics are similar to those of *f. typica*.

Note on the three forms of *Allolobophora japonica*

MICHAELSEN ('92) and BEDDARD ('95) have distinguished the present species into two forms. MICHAELSEN described as follows in his paper: „Die von Enosima stammenden Stücke sind klein, höchstens 42 mm lang und $2\frac{1}{2}$ bis 3 mm dick. Die Segmentzahl schwankt bei ihnen zwischen 96 und 126. Dabei sind sie fast farblos. Die Stücke von Hakodate dagegen sind gut mittelgross. Das Maximum ist 130 mm Länge, $5\frac{1}{2}$ Dicke und eine Zahl von 155 Segmenten. Sie sind ziemlich dunkel, rötlich gefärbt.“ BEDDARD's account is almost identical with that given by MICHAELSEN; the former writer might have made the description basing on the same specimens observed by the latter, and which were collected from Enosima and Hakodate.

In 1934 this species was separated into three forms of *f. typica*, *f. gigantica* and *f. minuta* by ÔISHI basing the descriptions on the specimens collected from various parts of Japan. They were defined by him as follows:

	<i>f. typica</i>	<i>f. gigantica</i>	<i>f. minuta</i>
Body-length -diameter	60-102 mm 3-5 mm	139-175 mm 5.2-7.2 mm	42-55 mm 1.75-2 mm
No. of segments	102-140	125-151	ca. 110
Body-colour	head pink, tail whitish-grey	dark reddish brown somewhat roundish	whitish grey
Pubertatis tubercles	triangular		roundish
Localities	Aomori Pref. (Asamusi, Ajigasawa, Mt. Hakkô- da), Sendai, Morioka, Tôkyô, Kôhu, Odawara, Nagano.	Tomakomai (Hokkaidô)	Morioka Asamusi

Judging from the descriptions given by MICHAELSEN and BEDDARD, „their small form“ may be identical with forma *typica* of ÔISHI and „their larger form“ with forma *gigantica* of the same.

The Korean specimens representing these forms are smaller than those found in Japan. Especially, those representing f. *gigantica* appear to be much smaller than the Japanese. But in the important characteristics such as the body-colour, the pubertatis tubercles etc., the Korean and Japanese specimens are closely identical. (However, on the Korean specimens of f. *gigantica* a further study is desirable as the examined ones are incomplete.) The differences in the size of setae existing among the three forms have not been mentioned by any of the foregoing writers.

From the descriptions given by other writers and also from the result obtained from the present study, the forms may be defined as follows:

	f. <i>typica</i>	f. <i>gigantica</i>	f. <i>minuta</i>
Body-length	12-102 mm	?-175 mm	24-55 mm
-diameter	2.5-5 mm	3.5-7.2 mm	1.75-2.8 mm
No. of segments	96-140	125-155	85-100
Body-colour	Head pink, tail whitish grey	darkish red	uniformly whitish grey
Pubertatis tubercles	triangular	triangular, but somewhat roundish	roundish
Size of setae	medium (0.39 mm × 36 μ)	large (0.54 mm × 47 μ)	small (0.29 mm × 24 μ)
Localities	Aomori Pref. (Asamusi, Ajigasawa, Mt. Hakkôda), Sendai, Morioka, Tôkyô, Kôfu, Odawara, Nagano, Kôrvô, Ryûjin, Enosima, Nikkô, Mt. Fuji?, Moji.	Tomakomai, Ak-kesi, Kwaizan, Hakodate, Mt. Fuji?, Moji?	Sendai, Asamusi, Ryûjin, Kôryô

8. *Bimastus parvus* EISEN 1874

1933 *Bimastus parvus*, CHEN, Contr. Biol. Lab. Sci. Soc. China, Zool. Ser., IX, 6, pp. 222-224, fig. 13.

1936 *Bimastus* sp., KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XI, 1, p. 183

1937 *Bimastus parvus*, ČERNOSVITOV, Rec. Ind. Mus., XXXIX, 2, p. 111.

1938 *Bimastus parvus*, GATES, Bull. Raff. Mus., Singapore, XIV, p. 222.

1938 *Bimastus parvus*, KOBAYASHI, Journ. Chôsen Nat. Hist. Soc., XXIV, p. 6.

1940 *Bimastus parvus*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XV, 3, p. 297.

Localities in Korea: N. Kankyô-dô: Kwainei, Kamisampô; S. Kankyô-dô: Genzan, Kôgen, Kankô, Hokusei; N. Heian-dô: Tetuzan, Teishû, Myôkôsan, Sin-anshû, Kyûjô, Kisen, Singishû; S. Heian-dô: Junsen, Yôtoku, Kaisen, Chôrin, Tinnampo, Heijô; Kôgen-dô: Tetugen; Keiki-dô: Monsan, Keijô, Ryûjin, Sin-i, Kôyô; S. Chûsei-dô: Gasan, Moshu; N. Zenra-dô: Riri, Nangen; S. Zenra-dô: Reisui; N. Keishô-dô: Kinsen.

9. *Bimastus beddardi* MICHAELSEN 1894

1900 *Helodrilus (Bimastus) beddardi*, MICHAELSEN, Tierreich, pp. 502-503.

1901 *Helodrilus (Bimastus) beddardi*, MICHAELSEN, Bull. Acad. Imp. Sci. St.-Petersburg, XV, 2, p. 213.

1902 *Helodrilus (Bimastus) beddardi*, MICHAELSEN, Jahrb. Hamb. Wiss. Anst., XIX, 2, p. 50.

1910 *Helodrilus (Bimastus) beddardi*, MICHAELSEN, Ann. Mus. Zool. Acad. Imp. Sci. St.-Petersburg, XV, p. 64.

1917 *Helodrilus (Bimastus) beddardi*, SMITH, Proc. U. S. Nat. Mus., LII, pp. 15-16.

1940 *Bimastus beddardi*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XV, 3, p. 298.

Localities in Korea and materials: Ryûjin, 1 clitellate specimen, VI/'34; Kôryô, Keiki-dô, 2 clitellate specimens, VII/'31.

Remarks: *B. parvus* and *B. beddardi* closely resemble each other. Possibly, their resemblance is apparent as shown by SMITH and also by the present writer, who remarks on this assumption in detail in his latest paper ('40). As he is still dubious with regard to the separation of these two species, in the present examination he has identified the specimens from the above mentioned localities with *B. beddardi*, basing the argument on their longer clitellar extension (fully occupying XXIII or XXIV-XXXI) and on their greater setal ratio. These characteristics only seem to distinguish this species from *B. parvus*.

10. *Bimastus tenuis* (EISEN) 1874

1938 *Bimastus tenuis*, KOBAYASHI, Annot. Zool. Japon., XVII, 3-4, p. 415.

Localities in Korea and materials: Hokusei, S. Kankyô-dô, 1 clitellate specimen, VIII/'35; Jôsin, N. Kankyô-dô, 23 clitellate specimens, VIII/'35.

Description: Colour in formalin, brownish-red dorsally, postclitellar region lighter than the preclitellar, pale ventrally, clitellum light flesh. The body-size is apparently constant; in most specimens, length 50-60 mm and greatest diameter 2.5-3.2 mm; the largest specimen measures 65 × 3.2 mm. Number of segments 100-103. Setae a and b of XVI are constantly planted on an oval papilla. Through the internal dissection they are found to be placed within the large setal sacs, and are grooved at about distal third; in one specimen the seta a measured about 0.56 mm in length and 26 μ in thickness at the proximal thickest portion. In only one specimen setae a and b of XXVI (left side only) were also planted on a similar papilla. Pubertatis tubercles are found on XXIX and XXX; they are well-developed, though rarely they are rather indistinct.

Spermathecae are absent (11 specimens were dissected).

A COMPARATIVE OBSERVATION OF THE HIND-BRAIN OF FISH POSSESSING BARBELS, WITH SPECIAL REFERENCE TO THEIR FEEDING HABITS¹

By

MITUO SATO

Hirosaki Middle School, Hirosaki, Aomori-Ken

(With 9 Text-figures.)

(Received November 8, 1940)

INTRODUCTION

Fish may be divided into two broad natural groups according to their feeding habits. One group is the surface-form or surface-feeder which seeks food mostly by sight at the surface of the water. The fish belonging to this group generally have large eyes. Another group is the bottom-form or bottom-feeder which feeds mainly by taste at the bottom or on rocky surface. In the fish belonging to this group the eyes are comparatively small and the taste-buds, no matter where they are located on the body, are largely responsible for detecting and picking out food from other substances in the water. According to HERRICK ('01, '02, '03, '06), the taste-buds in the pharynx and back parts of the mouth are supplied by the glossopharyngeal and vagus nerves, and those on the snout and outer surface of the skin are supplied by the facial nerves. The terminal centres of the nerve fibres of the vagus and facial nerves respectively are the vagal and facial lobes. Both these lobes form the important parts of the medulla oblongata. The size of these two lobes appears to depend upon the extent to which their nerve fibres supply the taste-buds. Accordingly, fish which detect food mainly by the taste-buds show enlarged vagal or facial lobes, namely the pattern of the hind-brain in the fish varies according to the feeding habits. This correlation between the structure of the hind-brain and the feeding habits has been confirmed by EVANS ('31, '35) and BHIMACHER ('35, '37).

In view of these results, it is interesting to observe the hind-brains of fish from the point of view of their relation to their feeding habits. In the bottom feeding fish the barbels are used for sorting out the edible

¹ This work was aided partly by a grant from the SAITÔ Gratitude Foundation.

substances. In addition to this, the barbel bears the majority of cutaneous taste-buds or terminal-buds (HERRICK, '01; LANDACRE, '07; SATÔ, '37d and others) which are innervated by the nerve fibres of the facial nerve. Consequently, it may be expected that the facial lobe will be prominent in such kinds of fish that use their barbels in order to search for food.

In the present paper, the writer attempted a naked eye examination of the hind-brains in the fish possessing barbels in order to confirm the correlation between the pattern of the hind-brains and their feeding habits. The histological anatomy of the hind-brain of these fish will be dealt with in a later paper.

Here the writer wishes to express his great appreciation of the advice and kind criticism given him by Prof. Dr. SANJI HÔZAWA.

RESULTS OF THE OBSERVATION

1. Cyprinoid fishes. The writer observed the brains of the following four species: *Cyprinus carpio* L., *Pseudogobio esocinus* (T. & S.), *Hemibarbus barbus* (T. & S.) and *Acheilognathus moriokae* JORDAN & THOMPSON.

Cyprinus carpio, the carp, as HERRICK (1899) and EVANS ('31) have already observed, shows prominent vagal lobes. The vagal lobes form a pair of wing-like swellings on either side of the medulla oblongata and embrace between their anterior ends the unpaired facial lobe (Fig. 1). The facial lobe is not so distinct.

Pseudogobio esocinus shows a similar pattern of hind-brain to that of the foregoing species, but the vagal lobes are somewhat smaller than those of the carp (Fig. 2).

In *Hemibarbus*, however, the vagal lobes pass back from the acoustic tubercles as narrow ridges and enclose the unpaired facial lobe which is moderate size (Fig. 3).

In *Acheilognathus moriokae* the vagal lobes are small and there is no well-defined facial lobe (Fig. 4). But, the optic lobe are very large as compared with these two lobes.

EVANS ('31) and BRIMACHER ('35), from their studies of the British and Indian cyprinoid fish, have divided these fish into the following three groups:

Group I. Fish that feed by taste, namely by the palatal organ (closely packed taste-buds in pharynx) which is innervated by the vagal nerve. Hence, there is a very prominent development of the vagal lobes in this

group.

Group II. Fish that use their barbels to sort out food on the bottom. They show an enlarged facial lobe.

Group III. Fish that feed largely by sight at the surface of water. They exhibit very small vagal facial lobes.

From the conclusions mentioned above and from the results obtained from the writer's observations, it may be expected that the carp, *Pseudogobio esocinus* and *Hemibarbus barbus* must be put into Group I and *Acheilognathus moriokae* into Group III. Now when we consider the feeding habits of these fish in their natural habitat, it is found that they subsist on a mixed diet, viz., they feed on worms, larvae of insects, some vegetable substances and so on. The carp and *Pseudogobio*, however, are mud-feeders, as shown

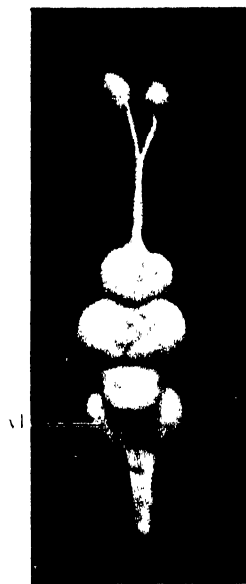


Fig. 1. The brain of the carp, as seen from above. (slightly enlarged). vl, vagal lobe.

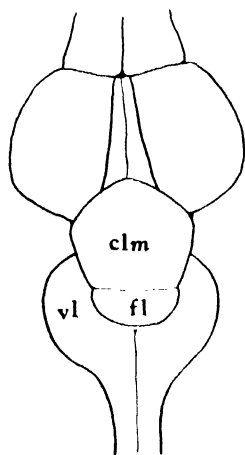


Fig. 2. Dorsal aspect of the brain of *Pseudogobio esocinus*. clm, cerebellum; fl, facial lobe; vl, vagal lobe.

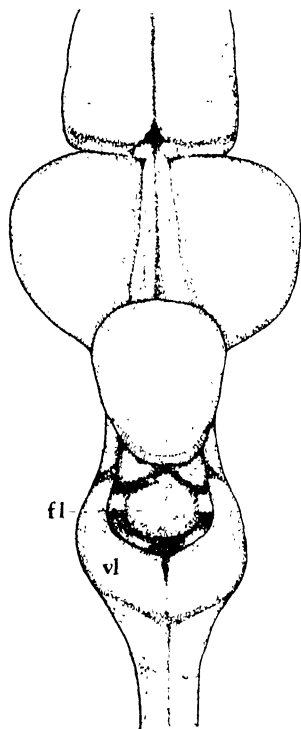


Fig. 3. Dorsal aspect of the brain of *Hemibarbus barbus*.

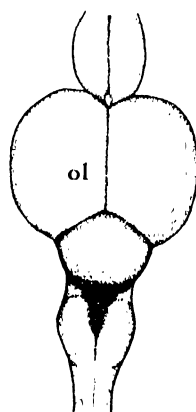


Fig. 4. Dorsal aspect of the brain of *Acheilognathus moriokae*. ol, optic lobe.

by EVANS ('31). These fish swallow mud directly into the mouth and sort out food from other substances by using the palatal organs found in the mouth. Accordingly, these two kinds of fish should belong to Group I. *Hemibarbus* has feeding habits similar to those of the carp, but it must be kept in mind that this fish often jumps on the surface of water. Therefore, this fish seems to detect food by sight and also by taste, in other words, this fish seems to possess characteristics of both surface- and mud-feeders. From the feeding habit and the pattern of the hind-brain, the writer is inclined to think that this fish may be placed in an exceptional case showing both the characteristics of Group I and III. In spite of the external character of the brain, *Acheilognathus moriokae* apparently

is never put into Group III the surface-feeders, because this fish never jumps. Moreover, judging from the contents in the digestive canal of this fish, it seems to have similar feeding habits to that of the carp. These evidences seem to favour the view that this fish may be placed in Group I. rather in Group III.

2. *Misgrunus anguillicaudatus* (CANTOR), the Loach. The loach shows a similar pattern of hind-brain to that of *Hemibarbus barbus*. But, the facial lobe is more prominent than in *Hemibarbus* and separates the anterior ends of the vagal lobes to a great extent (Fig. 5).

Furthermore, this fish detects edible substances mainly by using the taste-buds on the barbels and on the outer skin of the body. Thus the loach must be placed in Group II.

3. Catfish. The following four species are under consideration :

Parasilurus asotus L., *Pelteobagrus nudiceps* (SAUVAGE), *Pseudobagrus auranticus* (T. & S.), and *Liobagrus reini* HILGENDROF. All these fish have very similar feeding habits. They are all bottom and nocturnal-feeders. In these fish, the eyes are probably of little use in feeding, as shown already by HERRICK ('02), PARKER ('10) and OLMSTED ('18). They detect food mainly by using the taste-sense - the cutaneous taste-buds on the barbels and on the outer skin of the body.

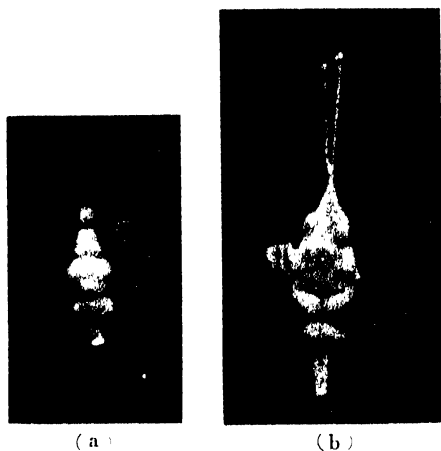


Fig. 5. Dorsal aspect of the brains of the loach (a) and *Plotosus anguillaris* (b). (natural size).

Associated with the feeding habits, the cutaneous gustatory organs are highly developed in these fish. This development has induced an enlargement of the facial lobes. Thus the pattern of the hind-brains in these fish are almost similar (Fig. 6 and 7). The bilateral facial-lobes are peculiarly developed and are placed immediately anterior to the vagals which are somewhat smaller than the facials. In all these fish the acoustic tubercles are well-marked and the cerebellum overlaps the optic lobes dorsally.

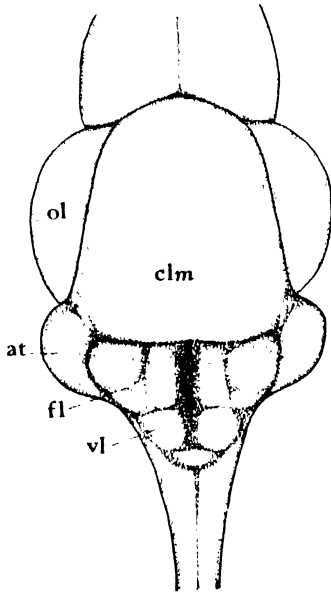


Fig. 6. Dorsal aspect of the brain of *Pelteobagrus nudiceps*.
at, acoustic tubercle.

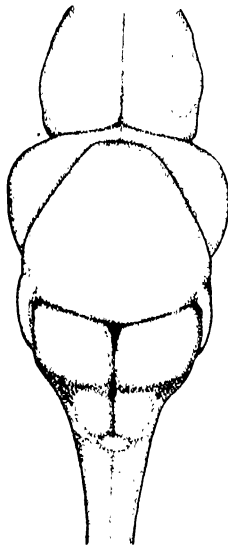


Fig. 7. Dorsal aspect of the brain of *Liobagrus reini*.

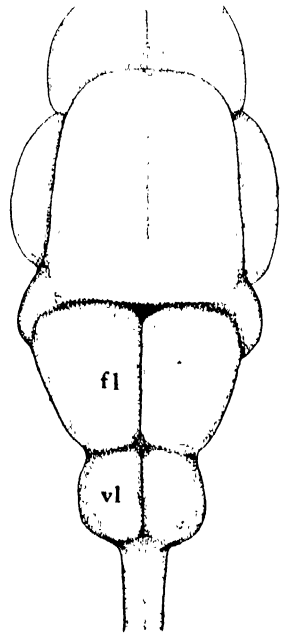


Fig. 8. Dorsal view of the brain of *Plotosus anguillaris*.

From these results, it may be concluded that the catfish should belong to Group II.

The sea catfish, *Plotosus anguillaris* (LACÉPÈDE) is placed in the "catfish group", because its brain character and feeding habits exhibit no remarkable deviation from that of the catfish mentioned above. The brain of this fish so closely resembles that of the catfish that it would be difficult to distinguish them externally (Fig. 5 and 8). This fish also is a typical bottom and nocturnal-feeder, and it detects, in which worms

and crustacea are found predominant, mainly by the cutaneous gustatory organs found on the barbels and snout (SATÓ, '37 c).

4. Goatfish. In the writer's previous papers (SATÓ, '37 a, b and '38), confirmed the function of the barbels and the feeding habits of the following two goatfish, *Upenoides bensasi* (T. & S.) and *Upeneus spilurus* BLEEKER and proved that these two fish live in the mud and search for their food exploring the bottom using their barbels. The barbels are the

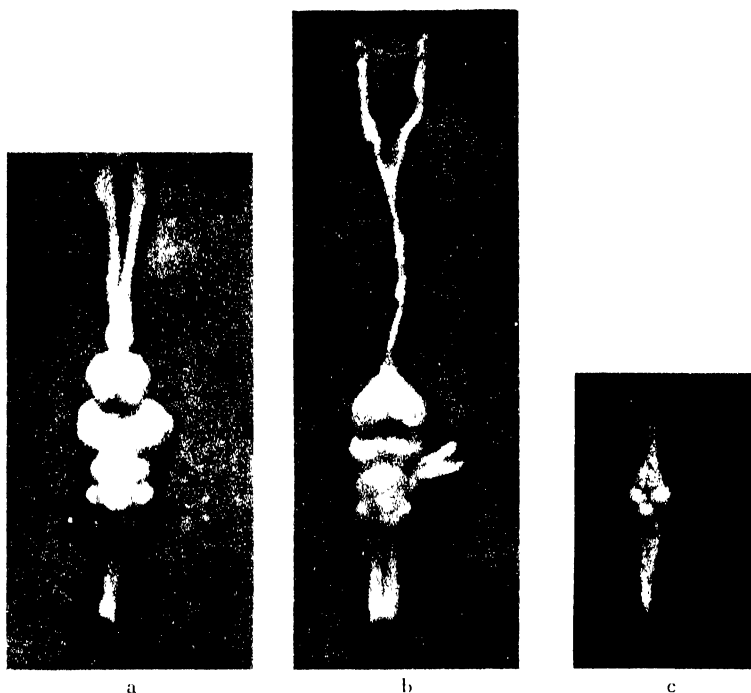


Fig. 9. Dorsal view of the brains of the following three fish (natural size).
a. *Upenoides bensasi*; b. *Brotula mutibarbata*; c. *Apistus evolans*.

notable characteristic seen at the chin and they bear a great number of cutaneous taste-buds. The goatfish feed mainly on worms in the mud and the barbels seem to be a limiting factor in sensing the worms.

Correlated with the feeding habits mentioned above, the facial lobes are well-marked on either side of the cerebellum (Fig. 9). In these fish, the facial lobes are more anterior in situation than those of the carp and the catfish. The vagal lobe is not so prominent, but to give its precise situation and that of the facial lobe needs further microscopic observation.

5. *Brotula mutibarbata* T. & S. This fish has twelve barbels about

the mouth. Fisherman say that it seems to be one of the bottom-feeders. The hind-brain bears resemblance to that of the foregoing species, the goatfish: the facial lobes being marked (Fig. 9). The cerebellum is comparatively large and overlaps the optic lobes dorsally.

6. *Apistus evolans* JORDAN & STARK. This fish remains most of time quietly on the bottom and thus seems to be a bottom-feeder. The pattern of the hind-brain, however, does not show any of the characteristics of the bottom-feeder. The vagal and facial lobes are difficult to discriminate with a naked eye observation (Fig. 9). It is hoped that further work on the microscopic anatomy of this fish will help to throw light upon these points when can be only examined incompletely by the naked eye.

SUMMARY

In preparing the present paper, the writer performed a naked eye examination on the hind-brains of 14 species of fish in relation to their feeding habits. All the fish under consideration have the barbels about the mouth. From the results obtained by the present observations these may be summarized as follows, after the manner of EVANS and BHIMACHER:

Group I. *Cyprinus carpio*, *Pseudogobio esocinus*.

All these fish are mouth-tasters, viz., they sort out food from the mud by help of the taste buds in the mouth. Hence, in these fish the vagal lobes are very developed.

Group II. *Misgrunus anguillicaudatus*, *Parasilurus asotus*, *Pelteobagrus nudiceps*, *Pseudobagrus aurantiacus*, *Liobagrus reini*, *Plotosus anguillaris*, *Upeneoides bensasi*, *Upeneus spilurus*, *Bortula multibarbata*.

All the members of this group have notable barbels about the mouth, and sort out the food on the bottom by aid of taste-buds found on the body surface, especially on the barbels. Throughout this group the facial lobe is very well-marked.

Group III. *Hemibarbus barbus*. This fish has both the characteristics of the surface-feeder and of the mud-feeder.

As summarized above, it is certain that the external characters of the hind-brain closely agree with the habits of feeding. But, it must be kept in mind that there are exceptional cases such as the *Acheilognathus morio* and *Apistus evolans*. The former shows a similar manner in its feeding habits to the carp, although the vagal lobe is very small. The latter is one of the bottom-feeders, but the vagal and facial lobes are

minute. On these points more precise information will be obtained by microscopic examination of the hind-brain.

REFERENCES

- BHIMACHER, B. S., 1935. A comparative anatomy of the correlation between the feeding habits and the structure of the hind brain in the South Indian Cyprinoid Fishes. *Proc. Royal Soc.*, Vol. 117. pp. 258-277.
- , 1937. A study of the medulla oblongata of Cyprinodont fishes with special reference to their feeding habits. *Ibid.* Vol. 123. pp. 59-68.
- EVANS, H. M., 1931. A comparative study of the brains in British Cyprinoids in relation to their habits of feeding, with special reference to the anatomy of the medulla oblongata. *Ibid.* Vol. 108. pp. 233-257.
- , 1935. The brain of *Gadus*, with special reference to the medulla oblongata and its variations according to the feeding habits of different Gadidae. *Ibid.* Vol. 117. pp. 367-399.
- HERRICK, C. J., 1899. The cranial and first spinal nerves of *Menidia*. *Arch. of Neur. and Psycho-pathol.*, Vol. 2. pp. 21-319.
- , 1901. The cranial nerves and cutaneous sense organ of the North American Siluroid Fishes. *Jour. Comp. Neurol.*, Vol. XI. pp. 174-249.
- , 1902. The organ and sense of taste in fishes. *Bull. U. S. Fish Commiss.*, Vol. 22. pp. 237-272.
- , 1903. On the Phylogeny and morphological position of the terminal buds of fishes. *Jour. Comp. Neurol.*, Vol. 13. pp. 121-138.
- , 1906. On the center for taste and touch in the medulla oblongata. *Ibid.*, Vol. 16. pp. 403-440.
- LANDACRE, F. L., 1907. On the place of origin and method of distribution of the taste-buds in *Ameiurus melas*. *Ibid.*, Vol. 17. pp. 1-66.
- OLMSTED, J. M. D., 1918. Experiments on the nature of the sense of smell in the common catfish, *Ameiurus*. *Amer. Jour. Physio.*, Vol. 46. pp. 443-458.
- PARKER, G. H., 1910. Olfactory reactions in fishes. *Jour. Exp. Zool.*, Vol. 8. pp. 535-542.
- SATÔ, M., 1937 a. Preliminary report on the barbels of a Japanese goat-fish, *Upeneoides bensasi* (T. & S.). *Sci. Rep. Tôhoku Imp. Univ. Bio.*, Vol. XI. pp. 259-264.
- , 1937 b. Further studies on the barbels of a Japanese goatfish, *Upeneoides bensasi* (T. & S.). *Ibid.*, Vol. XI. pp. 297-302.
- , 1937 c. On the barbels of a Japanese sea catfish, *Plotosus anguillaris* (LACÉPÈDE). *Ibid.*, Vol. XI. pp. 323-332.
- , 1937 d. Histological observations on the barbels of fishes. *Ibid.*, Vol. 12. pp. 265-276.
- , 1938. The sensibility of the barbel of *Upeneus spilurus* BLEEKER, with some notes on the schooling. *Ibid.*, Vol. 12. pp. 489-500.

THE FRESH-WATER SPONGES OF TISIMA-RETTÔ (THE KURILE ISLANDS)

By

NOBUO SASAKI

Biological Institute, Tôhoku Imperial University, Sendai, Japan

(With Plates VII-IX and 6 text-figures)

(Received November 9, 1940)

The present paper deals with the fresh-water sponges obtained from the Southern and the Northern Kurile Islands. Owing to the kindness of Dr. D. MIYADI and Dr. M. UENO, the writer has recently had the opportunity of examining the sponges which these gentlemen have generously sent to him. The specimens were collected from Kunasiri-sima (Kunashir Island) and Etorohu-sima (Iturup Island) of the Southern Kuriles and from Paramusiru-tô (Paramushir Island) and Simusyu-tô (Shumshir Island) of the Northern Kuriles by Dr. D. MIYADI, Mr. K. Koba, Viscount A. TANAKA and Mr. R. Hosino, during the years extending from 1932 to 1934. Acknowledgments are also due to Viscount A. TANAKA, Mr. R. Hosino and Mr. K. Koba who collected some parts of these valuable specimens. The writer also wishes to express his hearty thanks to Professor Dr. S. HÔZAWA for his kind directions and helpful advice.

A. The Fresh-water Sponges obtained from Minami-Tisima (the Southern Kuriles)

The fresh-water sponges obtained from Kunasiri-sima (Kunashir Island) and from Etorohu-sima (Iturup Island) were briefly reported upon by D. MIYADI in 1933.

The present writer would like here to deal more precisely with these sponges.

I. On the specimens obtained from Kunasiri-sima (Kunashir Island)

The fresh-water sponges of Kunasiri-sima were first investigated by MIYADI and were reported upon briefly by him in 1938. He had made his collection with help from the fund from the Keimei-kwai of Tôkyô. The specimens were obtained from the three lakes of Yanbetu-numa, Kunasiri-onnetô and Kunasiri-pontô by Viscount A. TANAKA and Mr. R.

HOSINO, and from the six lakes of Tôhutu-ko, Tôhutu-pontô, Hurukamappu-numa, Higasi-birokuko, Higasiburoku-pontô and Nikisiro-ko by Dr. D. MIYADI.

The following is the list of the species and variety taken from these lakes.

1. *Spongilla lacustris* (L.)
2. *Spongilla fragilis* LEIDY
3. *Ephydatia mülleri* var. *kunasiri*, n. var.

1. *Spongilla lacustris* (L.)

(Pl. VII, Figs. 1, 2)

Spongilla lacustris, LINNÉ 1759, p. 1348.

Spongilla lacustris, CARTER 1881, p. 87; POTTS 1887, p. 186; ANNANDALE and KAWAMURA 1916, p. 3; SCHRÖDER 1932, p. 127; SASAKI 1934, p. 219, 1939 p. 120.

Localities.—Higasibiroku-ko, Yanbetu-numa?, Kunasiri-pontô?, Tôhutu-ko?, Higasibiroku-pontô?.

Remarks.—Of the specimens those from Higasibiroku-ko only possess gemmules, while those from the remaining four lakes affixed with question marks possess none. But judging from the characteristic external forms and the flesh-spicules, these specimens seem to indicate the present species as being quite distinct from others found in this island. The specimens from Higasibiroku-ko belongs to *Spongilla lacustris* forma *typica* having thick-walled gemmules provided with a single foraminal aperture and abundant gemmule-spicules. The measurements of the sponge are shown in the next paragraph. The skeleton-spicules are 210–380 μ (average 295.4 μ) long and 8–16 μ (average 12.84 μ) thick at the thickest portion. The flesh-spicules are 56–96 μ (average 77.5 μ) long and 4–8 μ (average 6.15 μ) thick at the thickest portion.

The gemmule-spicules are 45–91 μ (average 68.55 μ) long and 4–6 μ (average 4.95 μ) thick in the thickest portion. The diameter of the gemmules measures 550–800 μ (average 704.0 μ) and the pneumatic covering of the same is 45–60 μ thick.

2. *Spongilla fragilis* LEIDY

(Pl. VII, Figs. 3, 4)

Spongilla fragilis, LEIDY 1851, p. 278; POTTS 1887, p. 197; ANNANDALE 1909, p. 106; ARNDT 1928, p. 60; SCHRÖDER 1932, p. 130; SASAKI 1934, p. 226, 1939, p. 126.

Localities.—Kunasiri-pontô, Higasibiroku-ko, Hurukamappu-numa?, Kunasiri-onnetô?.

Remarks.—No gemmules were found in the specimens taken from Hurukamappu-numa and from Kunasiri-onnetô, thus their identification seems to be somewhat uncertain.

The specimens from Kunasiri-pontô and from Higasibiroku-ko have many characteristic gemmules. The gemmule-spicules in these two specimens are in most cases rounded at both ends.

Measurement.

Specimen from Kunasiri-pontô:	Length (average)	Diameter measured at the thickest portion (average)
Skeleton-spicules	210-410 μ (339.8 μ)	9-21 μ (16.78 μ)
Gemmule-spicules	70-115 μ (86.3 μ)	5-8 μ (6.1 μ)
Specimen from Higasibiroku-ko:		
Skeleton-spicules	220-360 μ (303.6 μ)	8-15 μ (12.9 μ)
Gemmule-spicules	67-145 μ (92.02 μ)	5-8 μ (6.3 μ)
Diameter of gemmules	280-450 μ (368.2 μ)	

3. *Ephydatia mülleri* var. *kunasiri*, n. var.

(Pl. VII, Figs. 5, 6, 7; Pl. IX, Fig. 41; Text-figs. 1, 2)

The description of this new sponge is based upon half disintegrated specimens.

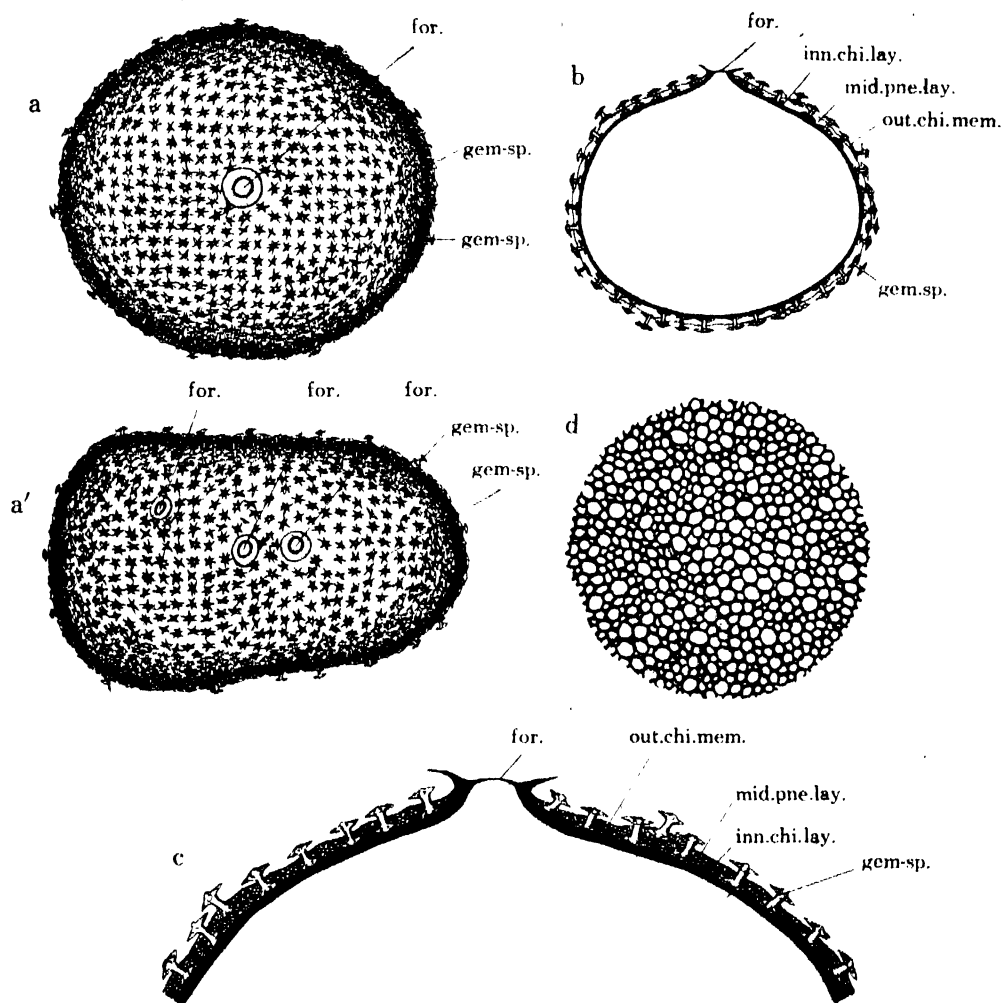
The external form of the sponge (Pl. VII, Figs. 5, 6, 7) may be said to represent either an irregular mass or a crust sometimes covered with a number of small projections.

The surface is uneven. Some of these specimens attain 2-3 cm. in diameter.

It is relatively hard and very brittle in consistency. The colour of the sponge when preserved in alcohol is grey, greyish-yellow or brown. The pores and oscula found on the surface are generally small and inconspicuous.

The skeleton is rather compact, the vertical fibres, each of which containing 5-8 or more spicules in its cross-section, are irregularly ramified, while the transverse fibres are poorly developed, containing 1-3 or more spicules in their cross-section, and are always dispersed among the vertical ones.

Gemmules (Pl. IX, Fig. 41: Text-fig. 1). The gemmules are formed within the interstices of the skeleton and are found specially abundant in the basal part of the sponge. The gemmules are very variable in both



Text-fig. 1. *Ephydatia mülleri* var. *kunasiri*, n. var. a, Gemmule, showing a foramen (=foraminal aperture) in the centre. a', Gemmule with three foraminal apertures. b, Section of a gemmule through the foramen. c, Sagittal section of the foramen. d, A part of the pneumatic layer. (a, a', b $\times 80$; c $\times 240$; d $\times 800$). for., foramen; gem-sp., gemmule-spicule; inn. chi. lay., inner chitinous layer; mid. pne. lay., middle pneumatic layer; out. chi. mem., outer chitinous membrane.

of shape and size (Text-fig. 1, a, a').

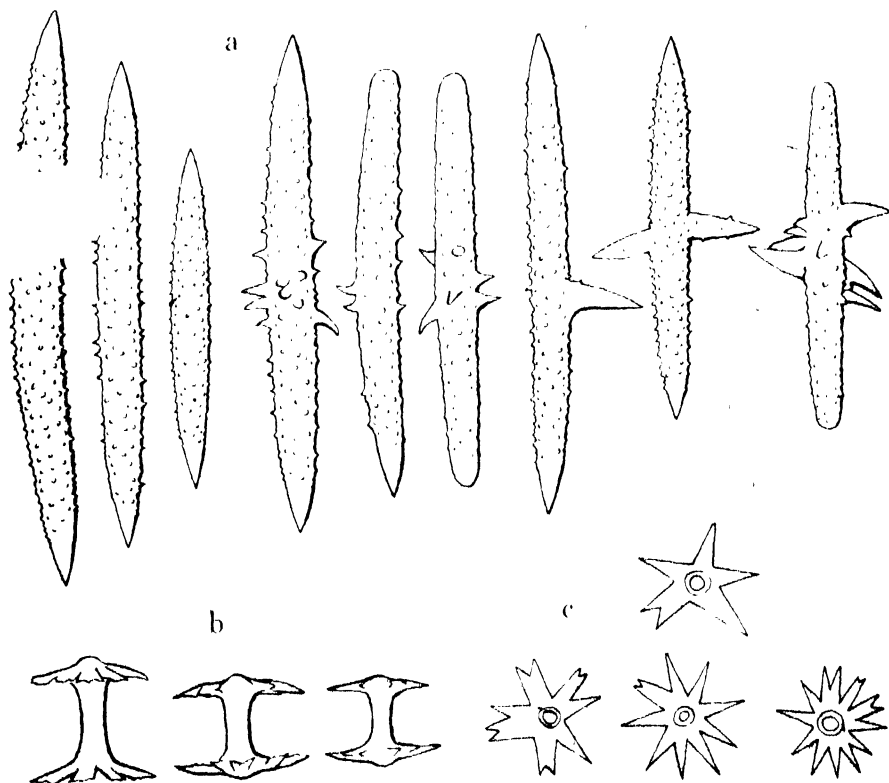
Generally they are spherical or crooked in form, measuring 400–780 μ (average 766.88 μ) in the greatest diameter.

Each gemmule is covered with a pneumatic coat (Text-fig. 1, b, c)

composed of three layers of 1) the inner chitinous layer which is $3-5\ \mu$ thick, 2) the middle pneumatic layer (Text-fig. 1, c, d) consisting of granular "ais-cells" of $5-10\ \mu$ thick and 3) the outer chitinous membrane which is sometimes very poorly developed being only $0.5-1\ \mu$ thick. In the coat above mentioned the birotulates are in most cases radially arranged in a single row, but sometimes one or more rows of the same kind of spicules may be added.

Generally each gemmule has a single foramen, but some have two or three (Text-fig. 1, a, a'). Ordinarily the foramen is protected by a shallow dish-like structure (Pl. IX, Fig. 41; Text-fig. 1, c), the diameter of which is $68-95\ \mu$ and the same of the inner operculum is $18-25\ \mu$.

Spicules (Text-fig. 2). The skeleton-spicules (Text-fig. 2, a) are rather stout, straight or slightly curved, sharply or abruptly pointed and sometimes



Text-fig. 2. *Ephedatia mülleri* var. *kunasiri*, n. var. a, Skeleton-spicules. b, Gemmule-spicules, side-view. c, Apical view of the rotules of the same. (a $\times 240$; b, c $\times 800$).

rounded at the extremities. There are some skeleton-spicules which are rounded at both ends, or sometimes rounded at one end and pointed at the other. As a rule, the skeleton-spicules are densely covered with minute spines excepting for the sharply pointed extremities.

Frequently these spicules have from 1 to 5 or more of comparatively large spines in the middle portion. The spines are sharply or abruptly pointed, smooth or beset with a few minute spines measuring 5-100 μ long and 5-20 μ thick at the thickest portion.

There are no flesh-spicules.

The gemmule-spicules (Text-fig. 2, b, c) are small birotulates. The shaft of the birotulate is usually shorter than the diameter of the rotule. It is short, stout, straight, smooth and measures 14-19 μ (average 16.76 μ) long and 3-4.5 μ (average 3.44 μ) in diameter. The rotules (Text-fig. 2, c) is flat, nearly smooth, deeply and irregularly serrated, forming 5-12 teeth. The rotules measure 17-25 μ (average 20.18 μ) in diameter.

Localities.—Tôhutu-pontô, Tôhutu-ko, Hurukamappu-numa and Nikisiro-ko.

Remarks.—*Ephydatia mülleri* var. *kunasiri*, n. var. is very closely allied to the typical form of the species as regards the form of the mule-spicules, and of the foramen etc. But this new variety differs from the typical *E. mülleri* in having a number of skeleton-spicules which are either rounded at one or at both extremities, and some are provided with from one to several large spines. This sponge seems to be harder than typical *E. mülleri* in consistency, having a more compact skeleton and thicker skeleton-spicules. The writer has named this new variety after the locality, Kunasiri-sima, in which the specimens were collected.

Measurement.—

Specimen from	Length	Diameter	Rotule diameter
	(average)	(average)	
Tôhutu-pontô:			
Skeleton-spicules	190-320 μ (272.7 μ)	17-26 μ (22.04 μ)	(average)
Gemmule-spicules	14-19 μ (16.76 μ)	3-4.5 μ (3.44 μ)	17-25 μ (20.18 μ)
Diameter of gemmules	480-780 μ (651.8 μ)		
Tôhutu-ko:			
Skeleton-spicules	240-290 μ (270.4 μ)	18-25 μ (22.08 μ)	
Gemmule-spicules	14-18 μ (16.05 μ)	3-4.5 μ (3.75 μ)	17-25 μ (19.85 μ)
Diameter of gemmules	400-580 μ (468.0 μ)		
Hurukamappu-numa:			
Skeleton-spicules	160-280 μ (231.5 μ)	14-25 μ (20.4 μ)	
Gemmule-spicules	14-18 μ (15.85 μ)	3-4.5 μ (3.625 μ)	18-25 μ (21.6 μ)
Diameter of gemmules	400-760 μ (592.6 μ)		

Specimen from	Length	Diameter	Rotule diameter
Nikisiro-ko:	(average)	(average)	
Skeleton-spicules	210-290 μ (246.6 μ)	13-20 μ (17.14 μ)	(average)
Gemmule-spicules	15-19 μ (16.5 μ)	3-4.5 μ (3.8 μ)	18-25 μ (22.3 μ)
Diameter of gemmules	430-750 μ (574.1 μ)		

II. On the specimens taken from Etorohu-sima (Iturup Island)

In 1933, the fresh-water sponges of Etorohu-sima were reported upon briefly by Dr. D. MIYADI who had collected the specimens with financial assistance from the Keimei-kwai fund during July-August of 1932 and of 1933. He reported that the fresh-water sponges from Tôro-numa, Seseki-numa, Syana-numa, Rubetu-numa, Rausu-numa, Tosimoe-ko, Rebun-numa, Kimonma-numa and Kamuikotan-numa are all to be identified with *Spongilla inarmata* ANNANDALE. Subsequently all the specimens obtained from the above mentioned nine lakes and which had been examined by Dr. D. MIYADI were kindly sent by him to the writer together with the specimens from Sibetoro-ko. The writer identified these specimens with the following forms. The identification mentioned above does not seem to be quite adequate.

1. *Spongilla lacustris* (L.)
2. *Spongilla fragilis* LEIDY
3. *Spongilla akanensis* SASAKI
4. *Ephydatia fluviatilis* var. *etorohuensis*, n. var.
5. *Ephydatia mülleri* (LIEBERKÜHN)

1. *Spongilla lacustris* (L.)

(Pl VII, Figs. 8, 9, 10, 11, 12, 13, 14, 15)

Localities.— Tôro-numa, Seseki-numa, Syana-numa, Rubetu-numa, Tosimoe-ko, Rausu-numa, Rebun-numa and Kimonma-numa.

Remarks.— The specimens collected from these lakes above mentioned are rather small, but bear some gemmules. They are all referable to *S. lacustris* forma *polyporis* having thin-walled gemmules provided with from 1 to 3 or more foraminal apertures and a few gemmule-spicules. *S. lacustris* forma *polyporis* is different from *S. inarmata* in the following points. First, the gemmule of this form is covered with a thin pneumatic coat on which generally a small number of gemmule-spicules are attached, while the gemmule of *S. inarmata* is covered with a thick pneumatic coat which has no gemmule-spicules, but which is enclosed in a regular network of macroscleres similar in form to the skeleton-spicules. Secondly,

the gemmule of the present form has from 1 to 3 or more foraminal apertures protected by a dish-shaped structure, while the gemmule of the latter species has a single foraminal tubule which is not conspicuously curved.

The specimen from Kimonma-numa has remarkably thinner skeleton-spicules, flesh-spicules and gemmule-spicules than those from the other six localities above mentioned.

Measurement.

Specimen from	Length (average)	Diameter measured at the thickest portion (average)
Toro-numa:		
Skeleton-spicules	190-360 μ (289.5 μ)	8-17 μ (13.7 μ)
Flesh-spicules	52-98 μ (78.55 μ)	4-6 μ (4.65 μ)
Gemmule-spicules	59-155 μ (90.85 μ)	4-6 μ (4.8 μ)
Seseki-numa:		
Skeleton-spicules	200-340 μ (298.5 μ)	8-18 μ (12.95 μ)
Flesh-spicules	60-104 μ (86.45 μ)	3-6 μ (4.45 μ)
Gemmule-spicules	60-135 μ (89.35 μ)	4-6 μ (4.6 μ)
Syana-numa:		
Skeleton-spicules	200-360 μ (288.5 μ)	8-16 μ (13.05 μ)
Flesh-spicules	66-100 μ (83.85 μ)	4-6 μ (5.35 μ)
Gemmule-spicules	65-135 μ (101.5 μ)	3-5 μ (4.5 μ)
Rubetu-numa:		
Skeleton-spicules	180-370 μ (295.5 μ)	10-18 μ (14.45 μ)
Flesh-spicules	65-105 μ (88.05 μ)	5-8 μ (6.55 μ)
Gemmule-spicules	45-130 μ (87.5 μ)	4-7 μ (5.1 μ)
Tosimoe-ko:		
Skeleton-spicules	200-350 μ (290.5 μ)	8-18 μ (14.35 μ)
Flesh-spicules	62-110 μ (90.75 μ)	4-8 μ (6.85 μ)
Gemmule-spicules	55-120 μ (87.15 μ)	4-8 μ (5.75 μ)
Rebun-numa:		
Skeleton-spicules	220-400 μ (313.5 μ)	8-18 μ (13.75 μ)
Flesh-spicules	61-105 μ (87.95 μ)	5-8 μ (6.15 μ)
Gemmule-spicules	45-120 μ (87.05 μ)	4-8 μ (5.85 μ)
Kimonma-numa:		
Skeleton-spicules	180-270 μ (236.2 μ)	3-7 μ (4.48 μ)
Flesh-spicules	58-100 μ (76.54 μ)	2-4 μ (2.94 μ)
Gemmule-spicules	40- 98 μ (70.85 μ)	2.5-4 μ (3.3 μ)
Specimen from	Diameter of gemmules (average) -	
Rubetu-numa:	470-810 μ (610.5 μ)	
Tosimoe-ko:	440-680 μ (576.5 μ)	
Rebun-numa:	480-700 μ (583.5 μ)	
Kimonma-numa:	580-750 μ (645.5 μ)	

2. *Spongilla fragilis* LEIDY

(Pl. VII, Figs. 16, 17)

Localities.—Seseki-numa, Syana-numa and Tosimoe-ko.*Remarks*.—The specimens from these localities are very small in size, but bear some gemmules. The gemmule-spicules are as a rule rounded at both extremities, none of these being pointed.*Measurement*.

Specimen from	Length	Diameter measured at the thickest portion
	(average)	(average)
Seseki-numa:		
Skeleton-spicules	190-350 μ (300.5 μ)	10-18 μ (14.25 μ)
Gemmule-spicules	62-95 μ (80.65 μ)	5-8 μ (6.35 μ)
Syana-numa:		
Skeleton-spicules	180-340 μ (281.5 μ)	8-17 μ (13.75 μ)
Gemmule-spicules	63-100 μ (83.55 μ)	5-7 μ (5.85 μ)
Tosimoe-ko:		
Skeleton-spicules	190-340 μ (284.5 μ)	8-18 μ (14.55 μ)
Gemmule-spicules	70-105 μ (84.65 μ)	5-8 μ (6.85 μ)

3. *Spongilla akanensis* SASAKI

(Pl. VIII, Fig. 18)

Spongilla akanensis, SASAKI 1934, p. 230.*Locality*.—Sibetoro-ko (the specimen was collected by Dr. D. MIYADI).*Remarks*.—In 1933, the writer collected this species from Lake Akan in Hokkaidô and the next year described it as a new species. Neither the specimen from Lake Akan or from Sibetoro-ko have gemmules. They agree in the following points: First, they are very hard in consistency as their skeleton is very compact forming an irregular network. Secondly the skeleton-spicules are larger and distinctly thicker than those of the specimens from Hondo. They are usually covered with very minute spines except at the two extremities, but sometimes they are entirely smooth. The skeleton-spicules in the specimen taken from Sibetoro-ko are 250-370 μ (average 324.4 μ) long and 22-38 μ (average 29.9 μ) thick at the thickest portion.4. *Ephydatia fluviatilis* var. *etorohuensis*, n. var.

(Pl. VIII, Fig. 19; Pl. IX, Fig. 42; Text-figs. 3, 4)

The specimen of this new variety was collected by Dr. D. MIYADI in 1933.

The sponge forms a mass (Pl. VIII, Fig. 19) of about 3 cm. diameter growing together with some Bryozoa (*Plumatella* sp.). It is rather hard but brittle in consistency.

The colour is white in alcohol. The oscula and pores are small and not conspicuous. The skeleton is relatively compact and forms of a very irregular network.

The vertical fibres are very well-defined, and 2-8 or more skeleton-spicules are observed in cross-section. The transverse fibres are poorly developed, dispersed among the vertical fibres, and 1-2 or more of skeleton-spicules are seen in cross-section.

Gemmules. (Pl. IX, Fig. 42; Text-fig. 3). The gemmules are freely set within the interstices formed among the skeleton-spicules, and are specially abundant in the basal portion of the sponge body. They are spherical or subspherical in form (Text-fig. 3, a), yellow or brown in colour and measure 380-620 μ (average 484.4 μ) in diameter.

They are usually covered with a rather thick pneumatic coat (Text-fig. 3, b) bearing the gemmule-spicules radially arranged. The pneumatic coat (Text-fig. 3, c) is composed of three layers; viz. the inner chitinous layer of 6-10 μ thick; the middle pneumatic layer composed of granular air-cells and of 20-30 μ thickness (Text-fig. 3, d); and the outer chitinous layer which is 2-5 μ thick.

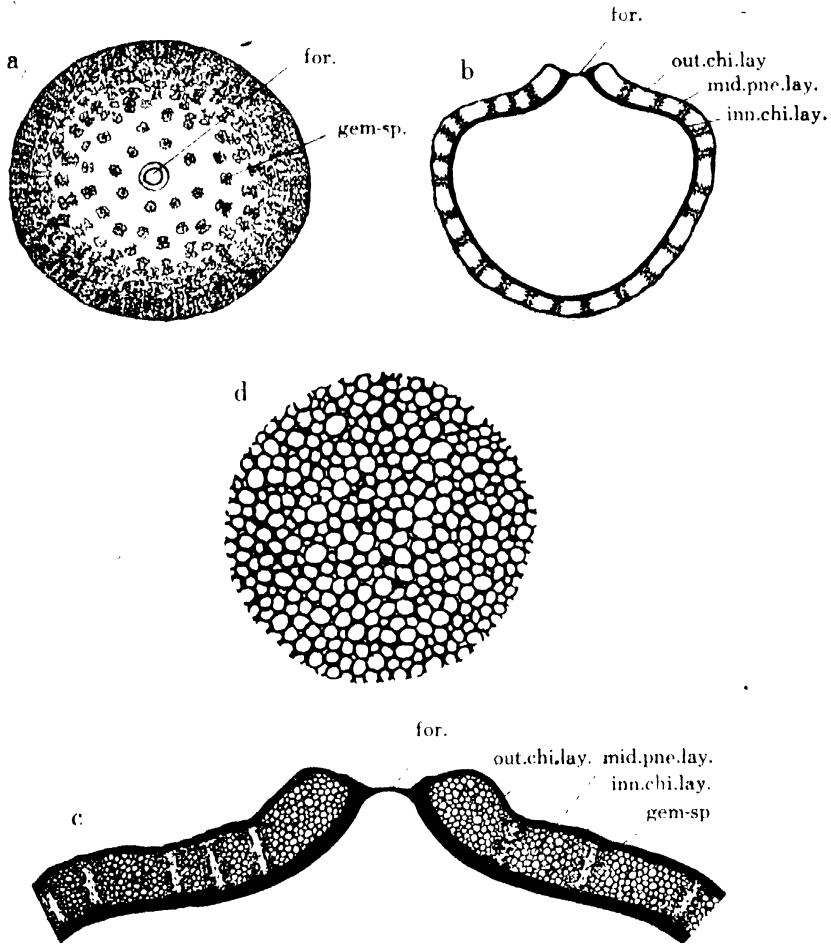
Each gemmule has a single foramen (Pl. IX, Fig. 42; Text-fig. 3, a, b, c) which is not protected by any remarkable structures, except for the chitinous operculum attached to the aperture. The diameter of the foramen is 40-50 μ and that of the chitinous operculum is 20-25 μ .

Spicules. (Text-fig. 4). The skeleton-spicules (Text-fig. 4, a) are slender, either straight or slightly curved, gradually and sharply pointed at both extremities.

As a rule, they are covered with very minute spines except at the two ends, while some spicules are entirely smooth. They are 220-380 μ (average 310.4 μ) long and 8-17 μ (average 13.16 μ) thick at the thickest portion.

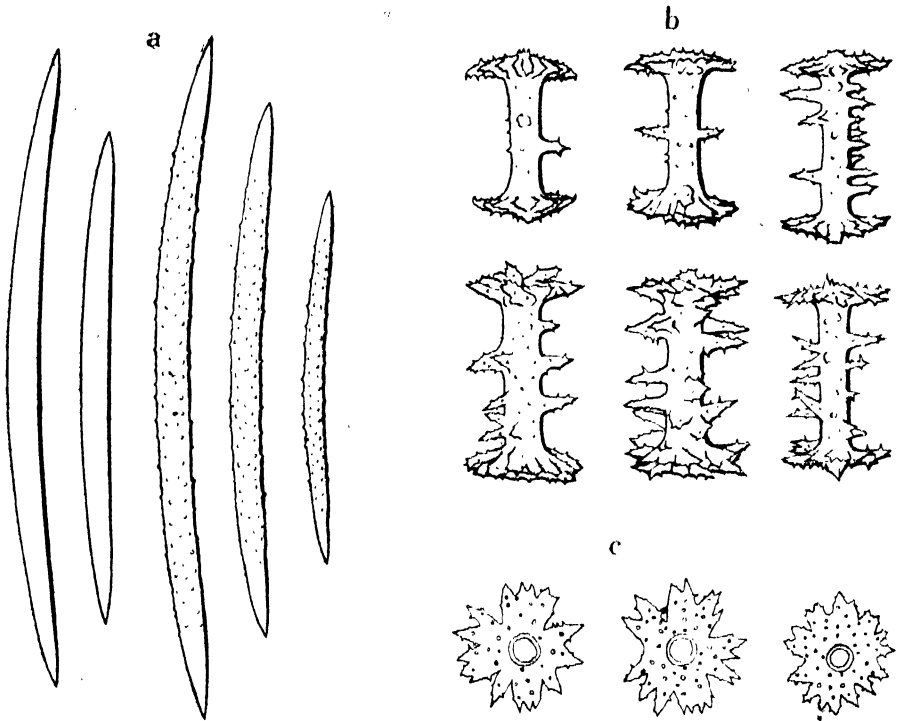
There are no flesh-spicules.

The gemmule-spicules (Text-fig. 4, b, c) are small birotulates. The length of the shaft is always longer than the diameter of the rotule. The shaft is straight, covered with a few minute spines, and, as a rule, is studded with from 1 to 12 or more large spines which are straight, stout, and sharply pointed. These spines are usually provided with a number



Text-fig. 3. *Ephydatia fluviatilis* var. *etorohuensis*, n. var. a, Gemmule, showing a foramen in the centre. b, Section of a gemmule through the foramen. c, Sagittal section of the foramen. d, A part of the pneumatic layer. (a, b $\times 80$; c $\times 240$; d $\times 800$). for., foramen; gem-sp., gemmule-spicule; inn. chi. lay., inner chitinous layer; mid. pne. lay., middle pneumatic layer; out. chi. lay., outer chitinous layer.

of minute spines. The shaft is $28-34\ \mu$ (average $30.12\ \mu$) long and $4-6\ \mu$ (average $4.74\ \mu$) thick at the thickest portion. The rotules are moderately serrated on the margin, and are covered with minute spines. They measure $17-24\ \mu$ (average $20.36\ \mu$) in diameter. Sometimes either the inner or the outer surface of the rotules is provided with from one to several micro-spinous spines. The spines are radially arranged and are nearly as



Text-fig. 4. *Ephydatia fluviatilis* var. *etorohuensis*, n. var. a, Skeleton-spicules. b, Gemmule-spicules, side-view. c, Apical view of the rotules of the same. (a $\times 240$; b, c $\times 800$).

long as the radius of the rotules. Thus they look like complex rotules when observed in side view.

Locality.-- Kamuikotan-numa.

Remarks.-- This new variety is closely allied to the typical *Ephydatia fluviatilis* (L.), if the form of the gemmules, the mode of arrangement of the gemmule-spicules, the form of the foramen, etc. are taken into consideration. But it differs from this species in having gemmule-spicules which are provided with several conspicuous large spines on the shaft and with complex rotules. In this new variety, the rotules and large spines of the gemmule-spicules are both provided with micro-spines. Such features as the spines on the shaft and on the rotules of gemmule-spicule, are also found in *Ephydatia subdivisa* (POTTS). But this new variety is different from *E. subdivisa* in the shape and in the size of the gemmule-spicules. *E. subdivisa* has much longer and thicker gemmule-spicules than

those of the present new variety. The gemmule of *E. subdivisa* resembles in appearance those of the members of genus *Heteromeyenid* to which the gemmule of this new variety bears no resemblance.

The writer has named this new sponge after the locality, Etorohusima, where the specimen was collected.

5. *Ephydatia mülleri* (LIEBERKÜHN)

(Pl. VIII, Figs. 20, 21)

Spongilla mülleri, LIEBERKÜHN 1856, p. 510.

Ephydatia mülleri, POTTS 1887, pp. 177, 224; ANNANDALE 1909, p. 116; ARNDT 1928, p. 68; SCHRÖDER 1932, p. 131; SASAKI 1934, p. 235.

Localities.—Tôro-numa, Syana-numa and Rausu-numa.

Remarks.—The specimens from Syana-numa and Rausu-numa have fairly thick skeleton-spicules. They are either straight or slightly curved, gradually and sharply pointed and are covered with minute spines. None of the skeleton-spicules have large spines in the middle portion nor are they rounded at the two extremities as in the case of the new variety, *Ephydatia mülleri* var. *kunasiri*, above mentioned.

Measurement.

Specimen from	Length of Skeleton-spicules.	Diameter measured at the thickest portion
	(average)	(average)
Tôro-numa:	190-300 μ (262.6 μ)	8-15 μ (12.64 μ)
Syana-numa:	230-290 μ (266.2 μ)	12-18 μ (13.82 μ)
Rausu-numa:	230-310 μ (276.2 μ)	13-22 μ (18.54 μ)

B. On the Fresh-water Sponges obtained from Kita-Tisima (the Northern Kuriles)

The fauna of the fresh-water sponges of Kita-Tisima (the Northern Kuriles) are up to the present almost unknown. In 1934, Dr. D. MIYADI made a limnological survey in Paramusiru-tô and Simusyu-tô of Kita-Tisima and collected some sponges of this group, specimens of which he later kindly sent to the writer for identification.

By the kindness of Dr. M. UENO the writer has also had the opportunity of examining an interesting specimen obtained from Paramusiru-tô by Mr. K. Koba in 1933.

III. On the specimens secured from Paramusiru-tô (Paramushir Island)

The specimens were collected in five localities in this island, they

represent three species only. Of these three, two are identical with species previously known, while the remaining one is here described for the first time. The list of the species is as follows.

1. *Spongilla lacustris* (L.)
2. *Spongilla paramusirensis*, n. sp.
3. *Ephydatia mülleri* (LIEBERKÜHN)

1. *Spongilla lacustris* (L.)

(Pl. VIII, Figs. 22, 23, 24)

Localities.—A small lake in Ura-yama near Murakami-wan, Lake No. 7 near Murakami-wan, a small lake near Siginosu-daira, a small lake near Suribati-wan.

Remarks.—The specimens taken from the localities above mentioned are all of the forma *polyporis* of *Spongilla lacustris*. That is they are provided with gemmules with a thin coat bearing a few gemmule-spicules and from 1 to 5 or more of foraminal apertures.

As shown in the next paragraph, the specimen from Lake No. 7 has comparatively thick spicules, while those from the remaining localities have fairly thin spicules.

Measurement.

Specimen from	Length of Skeleton-spicules. (average)	Diameter of the same measured at the thickest portion (average)
Ura-yama:	180-310 μ (259.7 μ)	5-9 μ (7.02 μ)
L. No. 7:	220-350 μ (278.3 μ)	8-20 μ (15.35 μ)
Suribati-wan:	190-300 μ (257.7 μ)	5-10 μ (7.65 μ)
Siginosu-daira:	190-320 μ (257.3 μ)	5-12 μ (9.25 μ)

2. *Spongilla paramusirensis*, n. sp.

(Pl. VIII, Fig. 25; Pl. IX, Figs. 43, 44; Text-figs. 5, 6)

The specimen of this new species was collected by Mr. K. Koba in July, 1933 and was kindly sent to the writer by Dr. M. Uéno.

In general, the sponge forms a relatively thin encrusting layer (Pl. VIII, Fig. 25) about two or three centimetres long, and not more than 5 millimetres in thickness.

The surface is rather even and smooth. The sponge is very soft but tenacious in consistency. The colour in alcohol is grey or pale brown.

The pores and oscula are present in abundance but are rather obscure on account of their small size. The dermal membrane is well-developed

and is provided with a number of free microscleres or flesh-spicules which are disposed without any orientation.

One of the most remarkable characteristics of this sponge is that the skeleton is composed of two different kinds of skeleton-fibres. One kind of these skeleton-fibres resembles those ordinarily found in other sponges of this group and is composed of a number of skeleton-spicules, from 2 to 10 or more of these being observed in cross-section.

The other kind is observed for the first time in this specimen. It is comparatively elastic and is usually seen to contain from 10 to 20 or more of very fine, hair-like spicules in its cross-section. These spicules are not so tightly bound together as seen in the case of the other fresh-water sponges, but they are loosely connected.

They are straight or slightly curved, smooth, very thin, sharply pointed at the extremities and are somewhat flexible (Text-fig. 6, d). They are easily dissolved in boiling acids or strong alkalis, thus they seem to be made up from spongin-like or horny substance. These spicules measure 170-240 μ (average 195.5 μ) in length and 1-1.5 μ (average 1.09 μ) thick at the thickest portion.

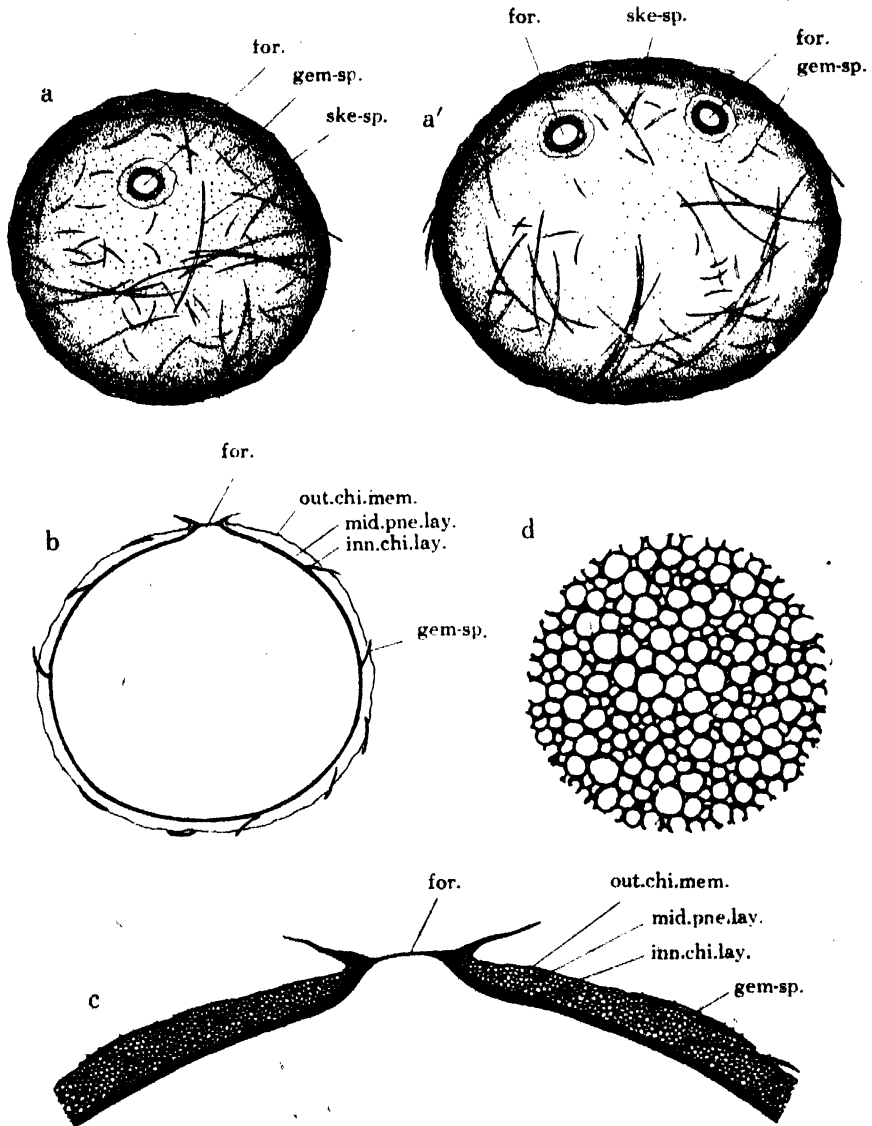
Gemmules (Pl. IX, Figs. 43, 44; Text-fig. 5). The gemmules are formed freely within the interstices of the skeleton, and are found specially abundant in the basal portion of the sponge body. They are spherical or subspherical in form (Text-fig. 5, a, a'), yellow or brown in colour, and are variable in size, being 310-700 μ (average 518.6 μ) in the greatest diameter.

Each gemmule is covered with a relatively well-developed pneumatic coat bearing the gemmule-spicules which are arranged tangentially or obliquely (Text-fig. 5, b, c).

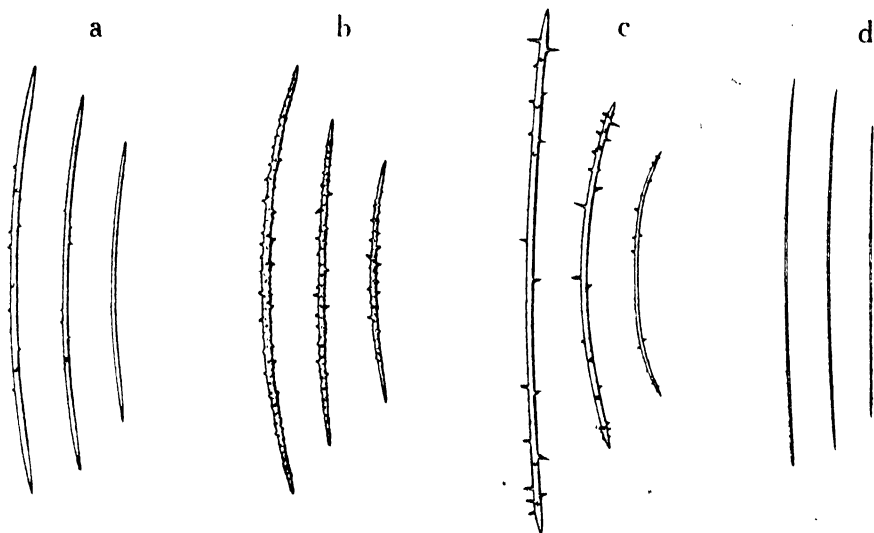
The pneumatic coat of the gemmule (Text-fig. 5, c) is usually composed of the following three layers, viz. 1) the inner chitinous layer which is 5-7 μ thick; 2) the middle pneumatic layer which is composed of granular air-cells (Text-fig. 5, d) and is 10-20 μ thick; and 3) the outer chitinous membrane which is usually undulated, and is 1-2 μ thick. As a rule, each gemmule has a single foramen but sometimes it has two of these (Text-fig. 5, a, a'). Usually the foramen is protected by a dish-shaped structure of 90-130 μ diameter (Pl. IX, Figs. 43, 44; Text-fig. 5, c).

The diameter of a foraminal aperture is 32-44 μ .

Spicules (Text-fig. 6). The skeleton-spicules (Text-fig. 5, a) are found to be very few in number, very small in size, very slender, either straight



Text-fig. 5. *Spongilla paramusirensis*, n. sp. a, Gemmule, showing a foramen in the centre. a', Gemmule with two foramina. b, Section of a gemmule through the foramen. c, Sagittal section of the foramen. d, A part of the pneumatic layer. (a, a', b $\times 80$; c $\times 240$; d $\times 800$). for., foramen; gem-sp., gemmule-spicules; inn. chi. lay., inner chitinous layer; mid. pne. lay., middle pneumatic layer; out. chi. mem., outer chitinous membrane; ske-sp., skeleton-spicule.



Text-fig. 6. *Spongilla paramusirensis*, n. sp. a, Skeleton-spicules. b, Flesh-spicules. c, Gemmule-spicules. d, Hairy spicules. (a, d $\times 240$; b, c $\times 800$).

or slightly curved, and gradually and very sharply pointed at both extremities. They are smooth or provided with a few minute spines, and measure 160–240 μ (average 209.1 μ) long and 2–4 μ (average 3.02 μ) thick at the thickest portion.

The flesh-spicules (Text-fig. 5, b) are small, very thin, straight or slightly curved, gradually pointed at both ends, densely covered with minute spines, and are 41–72 μ (average 55.06 μ) long and 1.0–1.5 μ (average 1.076 μ) thick at the thickest portion.

The gemmule-spicules (Text-fig. 5, c) are very slender, straight or curved, sharply pointed at both extremities, and are provided with a small number of straight spines.

They are very few in number and are 42–88 μ (average 58.4 μ) long and 0.5–1.2 μ (average 0.912 μ) thick at the thickest portion.

Locality.—Lake No. 3 near Banzyô-saki.

Remarks.—This new species may be easily distinguished from the other members of the same genus by the following points. First, it is very soft and elastic in consistency somewhat resembling a piece of cotton. Secondly, the skeleton is composed of two different kinds of fibres; one consisting of several skeleton-spicules when observed in their cross-section as is common in other sponges of this group, and the other consisting

of a number of very thin, characteristic spicules which have not hitherto been found in other fresh-water sponges. Thirdly, the skeleton-spicules, flesh-spicules and gemmule-spicules are much thinner than those of the other forms of fresh-water sponges found in Hondo. This new sponge somewhat resembles *Spongilla lacustris* (L.) in the shape of flesh-spicules, gemmule-spicules and in the features of the foramen, but differs from it in having micro-spined skeleton-spicules, and much thinner spicules.

3. *Ephydatia mülleri* (LIEBERKÜHN)

(Pl. VIII, Fig. 26)

Locality. — A small lake in Ura-yama near Murakami-wan.

Remarks. — The specimen is very small, attaining to only half a centimetre. Their spicules are comparatively thin as shown in the following.

Measurement. — The length of the skeleton-spicules is $200\text{--}320\ \mu$ (average $261.6\ \mu$) and $8\text{--}15\ \mu$ (average $11.92\ \mu$) thick at the thickest portion.

IV. On the specimens obtained from Simusyu-tô (Shumshir Island)

The specimens were collected from fifteen lakes and ponds in this island by Dr. D. MIYADI, and three different species were recognised, they are shown in the following list.

1. *Spongilla lacustris* (L.)
2. *Ephydatia fluviatilis* (L.)
3. *Ephydatia mülleri* (LIEBERKÜHN)

1. *Spongilla lacustris* (L.)

(Pl. VIII, Figs. 27-38)

Localities. — Lake No. 1, Lake No. 2 and Lake No. 3 in Hyakuikehara; C-ike in Bettobu; K-ike, Lake No. 6, Lake No. 7, Lake No. 8 and Lake No. 9 in Tenzin-yama; Azarasi-ike (Lake No. 10); O-ike, P-ike, Q-ike, Lake No. 13 and Lake No. 14 in Mituka-yama.

Remarks. — The specimens collected from the above mentioned localities are all examples of *Spongilla lacustris* forma *polyporis*. This form has gemmules with a thin pneumatic coat having a few gemmule-spicules, and with from one to several or more of foraminal apertures. Some of the gemmules found in the specimen from O-ike were found to have twenty or more foraminal apertures.

Measurement.

Specimen from	Length of skeleton-spicules.	Diameter of the same measured at the thickest portion.
	(average)	(average)
Lake No. 1:	180-290 μ (265.6 μ)	5-12 μ (8.85 μ)
Lake No. 2:	180-310 μ (260.3 μ)	6-12 μ (8.75 μ)
Lake No. 3:	160-300 μ (260.6 μ)	6-12 μ (9.95 μ)
C-ike:	180-310 μ (268.6 μ)	5-14 μ (11.05 μ)
K-ike:	160-300 μ (266.3 μ)	5-10 μ (7.05 μ)
Lake No. 6:	200-350 μ (289.7 μ)	8-14 μ (11.25 μ)
Lake No. 7:	170-330 μ (262.3 μ)	5-14 μ (9.35 μ)
Lake No. 8:	180-330 μ (276.7 μ)	5-13 μ (9.45 μ)
Lake No. 9:	180-310 μ (270.3 μ)	5-12 μ (9.35 μ)
Azarasi-ike:	160-320 μ (261.3 μ)	5-14 μ (9.40 μ)
O-ike:	170-330 μ (264.3 μ)	7-14 μ (9.35 μ)
P-ike:	180-350 μ (279.7 μ)	6-15 μ (10.05 μ)
Q-ike:	170-370 μ (284.3 μ)	8-15 μ (12.55 μ)
Lake No. 13:	180-340 μ (272.3 μ)	7-13 μ (9.85 μ)
Lake No. 14:	190-330 μ (267.3 μ)	6-13 μ (9.55 μ)

2. *Ephydatia fluviatilis* (L.)

(Pl. VIII, Fig. 39)

Spongia fluviatilis, LINNÉ 1759, p. 1348.*Ephydatia fluviatilis*, GRAY 1867, p. 550; ARNDT 1928, p. 66; SCHRÖDER 1932, p. 133; SASAKI 1934, p. 232.*Locality.*—Lake No. 9 in Tenzin-yama.

Remarks.—The specimen of the present species which was collected together with that of *Ephydatia mülleri*, is a very small and fragmental one, provided with a few gemmules. It has more slender skeleton-spicules and smaller gemmule-spicules than the other specimens of the same species hitherto collected from Hondo.

Measurement.—The skeleton-spicules are measured 180-350 μ (average 306.6 μ) long and 5-12 μ (average 8.67 μ) thick at the thickest portion.

The gemmule-spicules are 20-25 μ (average 22.1 μ) long. The diameter of the rotule is 11-17 μ (average 14.6 μ) and the same of the shaft is 1.5-3 μ (average 2.15 μ).

3. *Ephydatia mülleri* (LIEBERKÜHN)

(Pl. VIII, Fig. 40)

Localities.—Lake No. 9 in Tenzin-yama; Lake No. 14 in Mituka-yama.

Remarks.—Both the specimens obtained from these localities are very small and are half disintegrated. Each of them has a small number of

gemmules and comparatively thin spicules, the measurements of these specimens are shown in the following.

Measurements.

Specimen from	Length	Diameter	Rotule diameter
Lake No. 9:	(average)	(average)	
Skeleton-spicules	180-350 μ (291.7 μ)	6-12 μ (9.85 μ)	(average)
Gemmule-spicules	17-20 μ (18.8 μ)	3.5-5 μ (4.33 μ)	20-25 μ (22.2 μ)
Lake No. 14:			
Skeleton-spicules	190-310 μ (271.3 μ)	5-12 μ (9.25 μ)	
Gemmule-spicules	13-17 μ (14.9 μ)	3-4 μ (3.55 μ)	19-24 μ (21.7 μ)

Key to the fresh-water sponges of Tisima-Rettô (the Kurile Islands)

- A. Gemmule-spicules rod-shaped, straight or curved, spined, without transverse discs at both extremities.....(Genus *Spongilla*)
 - (a). Skeleton-spicules entirely smooth.
 1. Flesh-spicules present; pneumatic coat of gemmule granular; foramen of gemmule dish-shaped*S. lacustris* (L.)
 2. Flesh-spicules absent; pneumatic coat of gemmule composed of large polygonal air-cells; foramen of gemmule tubular*S. fragilis* Leidy.
 - (b). Skeleton spicules smooth or microspinous.
 3. Sponge very soft; skeleton-spicules small and very thin; flesh-spicules present, very thin*S. paramusirensis*, n. sp.
 4. Sponge very hard; skeleton-spicules big; flesh-spicules absent*S. akanensis* SASAKI.
- B. Gemmule-spicules with transverse discs at both extremities(Genus *Ephydatia*)
 - (c). Shaft of birotulates longer than rotule diameter.
 5. Gemmule-spicules rather smooth; rotules simple*E. fluviatilis* (L.)
 6. Gemmule-spicules very spinous; rotules complex*E. fluviatilis* var. *etorohuensis*, n. var.
 - (d). Shaft of birotulates as long as or shorter than rotule diameter.
 7. Skeleton-spicules microspinous, sharply pointed at both extremities*E. mulleri* (LIEBERKÜHN).
 8. Skeleton-spicules have or haven't large spines, pointed or rounded at both extremities*E. mulleri* var. *kunasiri*, n. var.

LIST OF REFERENCES

- ANNANDALE, N., (1909). Report on a collection of fresh-water sponges from Japan. Annot. Zool. Japonenses, Vol. 7, pp. 105-112.
- and KAWAMURA, T., (1916). The sponges of Lake Biwa. Journ. Coll. Sci. Imp. Univ. Tokyo, Vol. 39, Art. 1, pp. 1-27.
- ARNDT, W., (1928). Porifera, Schwämme, Spongien. Die Tierwelt Deutschlands, Jena. Teil 4, pp. 1-94.
- BOWERBANK, I. S., (1863). A monograph of the Spongillidae. Proc. Zool. Soc. London, pp. 440-472.
- CARTER, H. J., (1881). History and Classification of the known species of *Spongilla*. Ann.

- Mag. Nat. Hist., (ser. 5) Vol. 7, pp. 77-107.
- GRAY, I. E., (1867). Note on the Arrangement of Sponges with Description of some New Genera. Proc. Zool. Soc. London, p. 550.
- LEIDY, I., (1851). *Spongilla fragilis*. Proc. Acad. Nat. Sci. Philadelphia, p. 278.
- LIEBERKÜHN, N., (1856). Zusätze zur Entwicklungsgeschichte der Spongillen. Arch. Anat. phys., pp. 496-510.
- LINNÉ, C. V., Systema Naturae. Holmiae, 10th edition, Vol. 2, p. 1348.
- POTTS, E.A. (1887). Contribution towards a Synopsis of the American Forms of Fresh water Sponges with Descriptions of those named by other Authors and from all parts of the World. Proc. Acad. Nat. Sci., Philadelphia, pp. 158-296.
- SASAKI N., (1934). Report on the Fresh-water Sponges obtained from Hokkaidô. Science Reports of the Tôhoku Imperial University, 4th Series, Biology, Vol. 9, No. 2 and 3, Sendai, Japan, pp. 219-247.
- SCHRÖDER, K., (1932). Monographie der schlesischen Süßwasserschwämme. Abhandlungen der Naturforschenden Gesellschaft zu Görlitz, Heft 3, Band 31, pp. 111-138.

EXPLANATION OF THE PLATES

PLATE VII.

- Fig. 1. *Spongilla lacustris* (L.); from Yanbetu-numa, Kunasiri-sima.
- Fig. 2. Same; from Kunasiri-pontô, Kunasiri-sima.
- Fig. 3. *Spongilla fragilis* LEIDY; from Kunasiri-pontô, Kunasiri-sima.
- Fig. 4. Same; from Higasibiroku-ko, Kunasiri-sima.
- Fig. 5. *Ephydatia mülleri* var. *kunasiri*, n. var.; from Tôhutu-pontô, Kunasiri-sima.
- Fig. 6. Same; from Hurukamappu-numa, Kunasiri-sima.
- Fig. 7. Same; from Nikisiro-ko, Kunasiri-sima.
- Fig. 8. *Spongilla lacustris* (L.); from Tôro-numa, Etorohu-sima.
- Fig. 9. Same; from Seseki-numa, Etorohu-sima.
- Fig. 10. Same; from Syana-numa, Etorohu-sima.
- Fig. 11. Same; from Rubetu-numa, Etorohu-sima.
- Fig. 12. Same; from Tosimoe-ko, Etorohu-sima.
- Fig. 13. Same; from Rebun-numa, Etorohu-sima.
- Fig. 14. Same; from Rausu-numa, Etorohu-sima.
- Fig. 15. Same; from Kimonma-numa, Etorohu-sima.
- Fig. 16. *Spongilla fragilis* LEIDY; from Syana-numa, Etorohu-sima.
- Fig. 17. Same; from Tosimoe-ko, Etorohu-sima.

(Figs. 1-17, all natural size).

PLATE VIII.

- Fig. 18. *Spongilla akanensis* SASAKI; from Sibetoro-ko, Etorohu-sima.
- Fig. 19. *Ephydatia fluviatilis* var. *etorohuensis*, n. var.; Sibetoro-ko, Etorohu-sima.
- Fig. 20. *Ephydatia mülleri* (LIEBERKÜHN); from Tôro-numa, Etorohu-sima.
- Fig. 21. Same; from Rausu-numa, Etorohu-sima.
- Fig. 22. *Spongilla lacustris* (L.); from Lake No. 7, Paramusiru-tô.
- Fig. 23. Same; from a small lake near Suribati-wan, Paramusiru-tô.
- Fig. 24. Same; from a small lake near Siginosu-daira, Paramusiru-tô.
- Fig. 25. *Spongilla paramusirensis*, n. sp.; from Lake No. 3 near Banzyô-saki, Paramusiru-tô.

- Fig. 26. *Ephydatia mülleri* (LIEBERKÜHN); from a small lake in Ura-yama near Murakami-wan, Paramusiru-tô.
- Fig. 27. *Spongilla lacustris* (L.); from Lake No. 1 in Hyakuike-hara, Simusyu-tô.
- Fig. 28. Same; from Lake No. 2 in Hyakuike-hara, Simusyu-tô.
- Fig. 29. Same; from Lake No. 3 in Hyakuike-hara, Simusyu-tô.
- Fig. 30. Same; from C-ike in Bettôbu; Simusyu-tô.
- Fig. 31. Same; from K-ike in Tenzin-yama, Simusyu-tô.
- Fig. 32. Same; from Lake No. 6 in Tenzin-yama, Simusyu-tô.
- Fig. 33. Same; from Lake No. 7 in Tenzin-yama, Simusyu-tô.
- Fig. 34. Same; from Lake No. 8 in Tenzin-yama, Simusyu-tô.
- Fig. 35. Same; from Azarasi-ike (Lake No. 10), Simusyu-tô.
- Fig. 36. Same; from O-ike in Mituka-yama, Simusyu-tô.
- Fig. 37. Same; from Q-ike in Mituka-yama, Simusyu-tô.
- Fig. 38. Same; from Lake No. 13 in Mituka-yama, Simusyu-tô.
- Fig. 39. *Ephydatia fluviatilis* (L.); from Lake No. 9 in Tenzin-yama, Simusyu-tô.
- Fig. 40. *Ephydatia mülleri* (LIEBERKÜHN); from Lake No. 14 in Mituka-yama, Simusyu-tô.

(Figs. 18-40, all natural size)

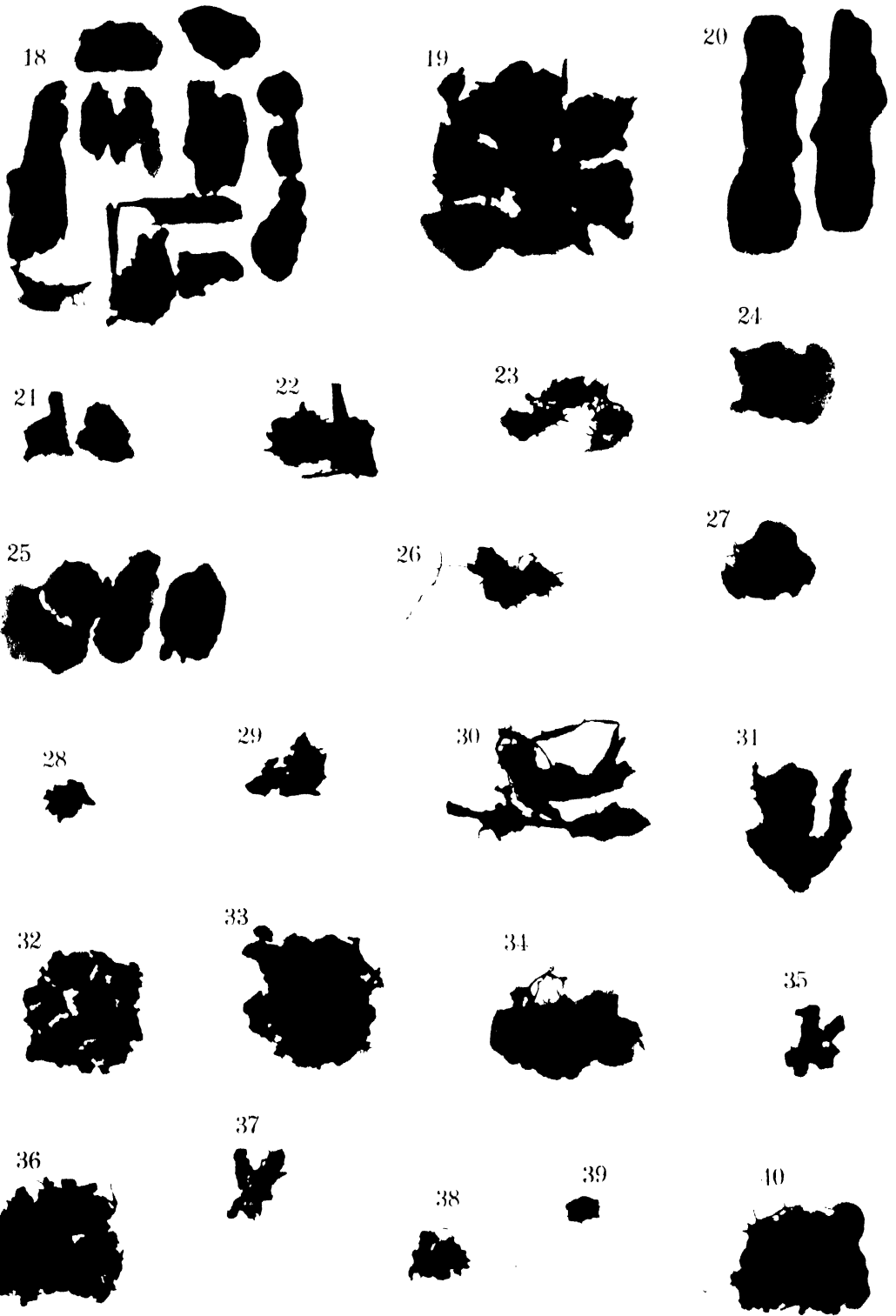
PLATE IX.

- Fig. 41. *Ephydatia mülleri* var. *kunasiri*, n. var.; from Tôhutu-pontô, Kunasiri-sima; Vertical section of the foramen of the gemmule.
- Fig. 42. *Ephydatia fluviatilis* var. *etorohuensis*, n. var.; from Sibetoro-ko, Etorohu-sima; Vertical section of the foramen of the gemmule.
- Fig. 43. *Spongilla paramusirensis*, n. sp.; from Lake No. 3 near Banzyô-saki, Paramusiru-tô; Vertical section of the foramen of the gemmule.
- Fig. 44. Same; Vertical section of the foramen of the another gemmule.

(Figs. 41-44, all $\times 300$).



SASAKI photo.



SASAKI photo.



SASAKI photo.

N. SASAKI: Fresh-water Sponges of Tisima-Rettô.

THE EMBRYOGENY OF *CUNNINGHAMIA* *LANCEOLATA* HOOKER

By

YOSINORI SUGIHARA

Biological Institute, Tôhoku Imperial University, Sendai

(With Plates X, XI and 2 text-figures)

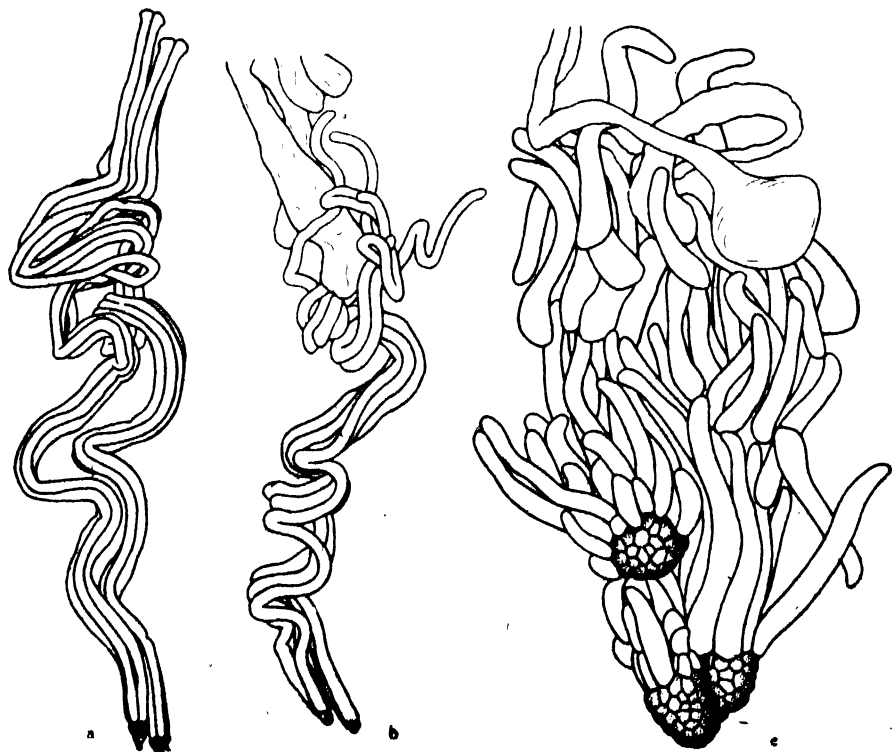
(Received November 14, 1940)

In the year 1910 MIYAKE published a paper dealing with the embryogeny of *Cunninghamia lanceolata*. He was concerned, however, mainly with the early stages of its development, the later stages not being investigated by him. So the present writer has examined the whole course of the embryogeny of this species. The results obtained are described in the present paper. The material was collected in 1939, from a cultivated tree in the grounds of the Sendai Forestry Office. The methods used are the same as those which the writer adopted in the previous investigations (SUGIHARA '39 '40).

The archegonia of *Cunninghamia lanceolata* are grouped together in a complex at the apex of the prothallium, and the complex, as was described by MIYAKE, has a sterile prothallial tissue at the centre. Two sperm-cells which are formed by the division of a body-cell are equal in size and shape.

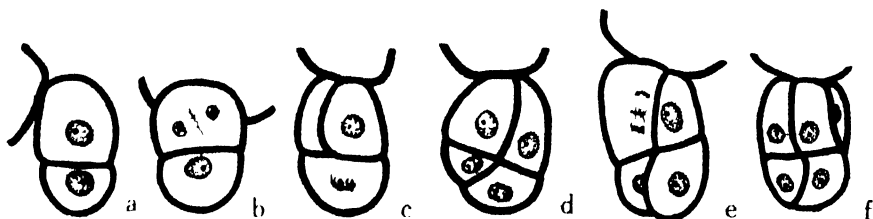
In 1939, the fertilization took place about the 20th of June in Sendai. Regarding the process of the fertilization and the formation of the pro-embryo, the writer's observation agrees well with that of MIYAKE. The contact of the male and the female nuclei takes place at the upper part of the archegonium, and they are wrapped in a granular cytoplasm (Pl. X, Fig. 1). They gradually pass down towards the lower end of the archegonium. The first mitosis of the fusion nucleus takes place near the middle of the archegonium. The spindle of the mitosis is very small in size, and the daughter nuclei are at first also very small. They are wrapped in a common granular cytoplasm (Pl. X, Figs. 2-3). After their arrival at the archegonial bottom both nuclei divide simultaneously and four nuclei are formed (Pl. X, Figs. 4-5). Then, the third division takes place also simultaneously and eight nuclei are formed. The wall-formation initiates in this stage (Pl. X, Fig. 6). The cells are arranged in two

tiers. The cells in the upper tier have no wall towards the archegonial cavity (Pl. X, Fig. 7). The number of the cells is, in most cases, four, five or six in the upper, and two, three or four in the lower tier. In the next stage the cells of the upper tier divide synchronously into two layers (Pl. X, Fig. 8). Thus three tiers of cells are formed, in which the cells of the uppermost tier are open to the archegonial cavity. The middle tier constitutes the prosuspensor. The lowest tier is the embryonic tier and divides once before the prosuspensor elongates (Pl. X, Fig. 9). The prosuspensor elongates windingly to an enormous length, but no separation of each prosuspensor cell, in general, takes place (Pl. XI, Fig. 11; Text-fig. 1, a). The cells in the embryonic tier are four to eight in number. Later in most cases, one or two of these cells situated at the apex become empty (Pl. XI, Figs. 12-13). These are the so-called cap-



Text-fig. 1. a, Advanced stage of the prosuspensor elongation. $\times 50$. b, Elongating stage of the primary suspensor. At the upper part of the figure the shrunken prosuspensor cells are seen. $\times 50$. c, Elongating stage of the embryonal tubes; the tips of the primary suspensor are swollen. $\times 70$.

cells. In the writer's observation, out of a total of fifty-seven cases sixteen had two empty cells at the apex and the remaining forty-one had each one empty cell. Whether there are any cases in which no cap-cell is formed is uncertain. In the next stage, each of the embryonic cells at the tip of the prosuspensor divides transversely (Text-fig. 2, a). Of the two daughter cells the one which lies next to the prosuspensor divides by a longitudinal wall (Text-figs. 2, b, c), while the one situated in the apical



Text-fig. 2. Embryonic units at the tip of the prosuspensor. $\times 320$. a, Two cell stage. b, Mitosis in the basal cell. c, Mitosis in the apical cell. d, Four cell stage. e, Additional mitosis in a basal cell. f, An exceptional case with four basal cells.

portion divides into four cells, generally disposing in a tetrahedral fashion (Pl. XI, Figs 14-16). Sometimes they are substituted by only two cells of unequal size, separated by an oblique wall. In the next stage, the two cells lying next to the prosuspensor elongate windingly like the prosuspensor (Pl. XI, Fig. 17; Text-fig. 1, b). They can be called the "primary" suspensor. In rare cases, one of the two cells ceases to elongate and degenerates. And in more rare cases, these two cells may be separated and grow independently with their own embryonic initial cells. As a result of the elongation of the primary suspensor the prosuspensor is pushed up into the archegonial region of the prothallium and gradually collapses. In the next stage, each of the embryonic cells at the apex of the primary suspensor begins to divide and to form a group of separated masses of embryonic cells. At this stage therefore the cleavage of the embryo takes place for the second time (Pl. XI, Figs. 18-19). The embryonic cells next to the primary suspensor later differentiate as the embryonal tubes, and finally a dicotyledonous embryo is produced (Pl. XI, Fig. 19; Text-fig. 1, c). Up to the present time the existence of the cap-cell is reported only in some species of *Podocarpus*, *Cephalotaxus*, *Araucaria*, *Agathis* and *Sciadopitys*. In these species, however, except in *Sciadopitys*, no cleavage polyembryony is found. So the cleavage polyembryony accompanied by the existence of the cap-cells in *Cunninghamia lanceolata*

is remarkable.

According to BUCHHOLZ's definition ('32), the primary suspensor is an elongated single cell in the basal portion of an embryo. But in *Cunninghamia*, as described above, the suspensor following the prosuspensor is constituted of two (sometimes three or four) (Text-figs. 2, e, f) elongated cells lying parallel to each other. If we accept BUCHHOLZ's definition, these cells cannot be considered as the primary suspensor. But, in the present writer's opinion, it seems more suitable to consider them as the primary suspensor on account of their exceptionally long elongation and their conspicuous difference from the following suspensor cells.

Concerning the chromosome number of this plant, MIYAKE ('10) reported the haploid number as twelve adduced from his observation of the meiosis of the pollen mother-cells. But the present writer clearly counted eleven bivalents in the meiotic metaphase in the pollen mother-cells. (Pl. X, Fig. 10).

SUMMARY

1) The writer's observation of the fertilization and formation of the proembryo of *Cunninghamia lanceolata* agrees with that of MIYAKE.

2) The components of the embryo system are the open cells, the prosuspensor, the primary suspensors, the embryonal tubes, the embryos proper and the cap-cells.

3) The primary suspensor of this plant is formed by two (sometimes three or four) elongated cells parallel to each other. These cells elongate windingly to an enormous length like the prosuspensor.

4) Cleavage polyembryony takes place twice; at the tip of the prosuspensor for the first time and later at the tip of the primary suspensor for the second time.

5) The haploid chromosome number of this species was estimated to be eleven.

Here the writer wishes to express his sincere gratitude to Professor Dr. M. TAHARA for his kind suggestions and criticisms during the course of this investigation.

LITERATURE

- BUCHHOLZ, J. T., 1932. The Suspensor of *Cryptomeria japonica*. Bot. Gaz., **93**: 221-226.
- MIYAKE, K., 1908. The Development of the Gametophytes and Embryogeny of *Cunninghamia*. (Preliminary Note). Bot. Mag, Tokyo, **22**: 45-50.
- MIYAKE, K., 1910. The Development of the Gametophytes and Embryogeny in *Cunninghamia sinensis*. Beih. Bot. Centbl., **27**, I: 1-25.
- SUGIHARA, Y., 1939. Embryological Observations on *Thujopsis dolabrata* var. *Hondai* MAKINO. Sci. Rep. Tôhoku Imp. Univ., 4th Ser., Biol., **14**: 291-303.
- SUGIHARA, Y., 1940. On the Multipartite Chromosome-Ring in *Cephalotaxus drupacea* SIEB. et Zucc. Sci. Rep. Tôhoku Imp. Univ., 4th Ser., Biol., **15**: 13-18.

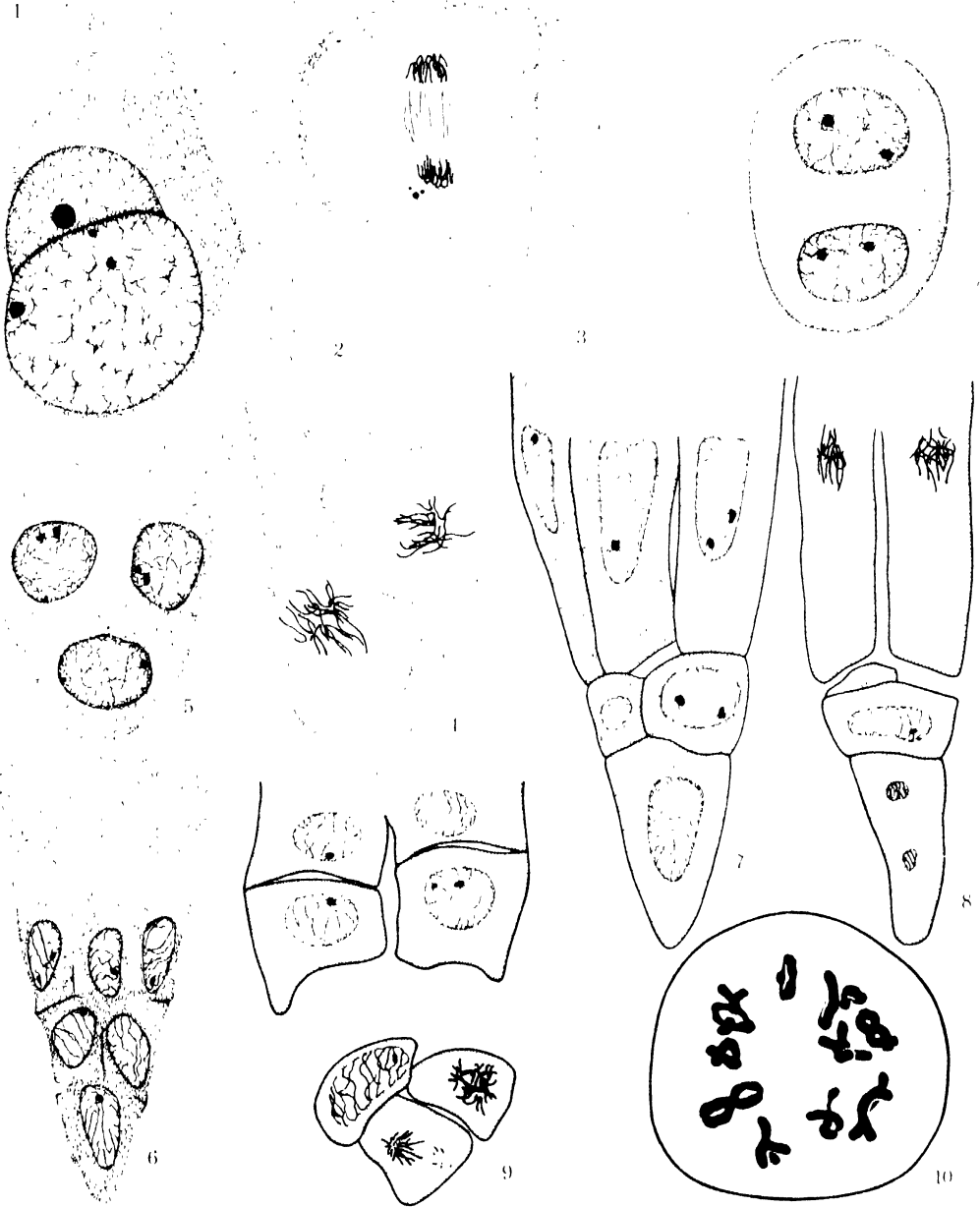
EXPLANATION OF PLATES

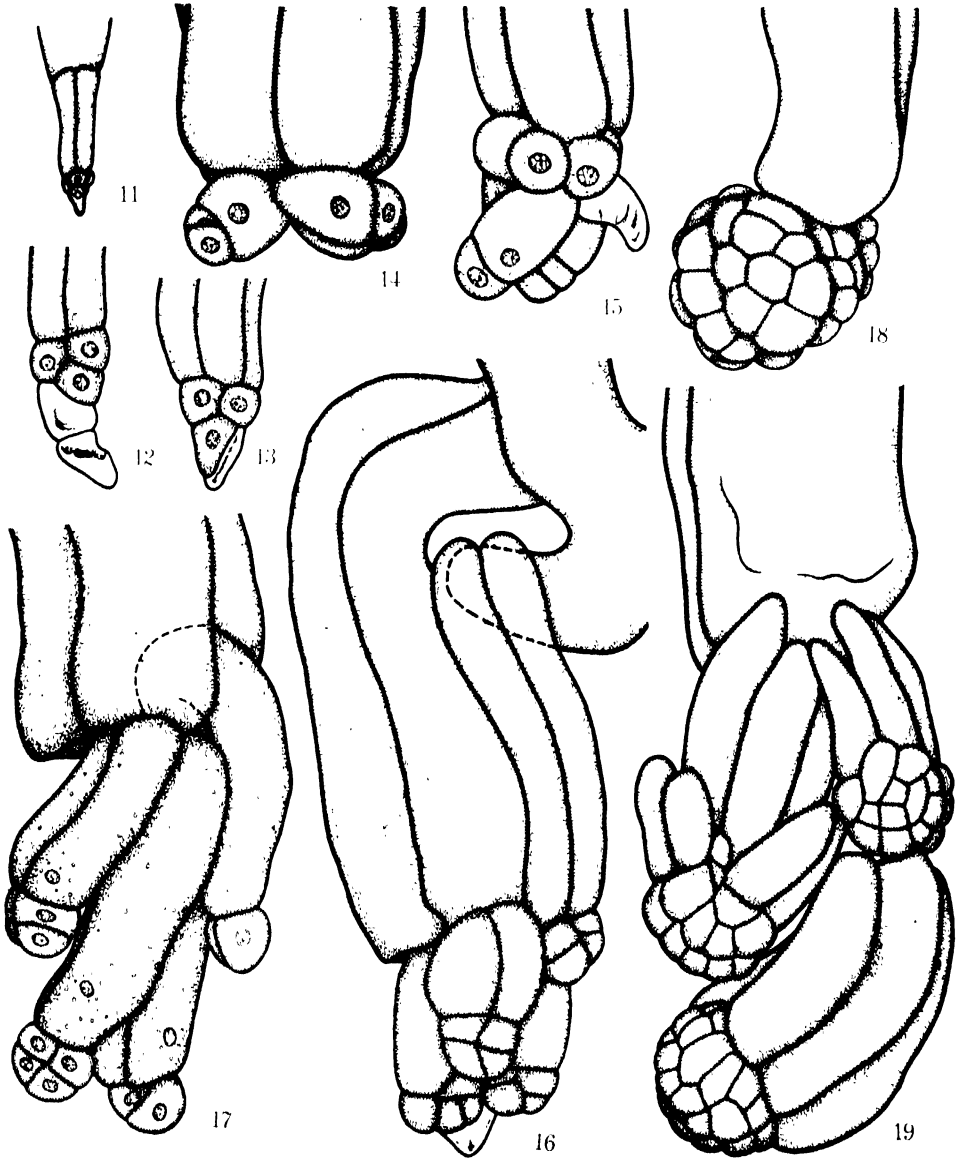
PLATE X.

- Fig. 1. Fertilization. $\times 480$.
Fig. 2. Anaphase of the proembryonal first division. $\times 480$.
Fig. 3. Two nuclei in a common granular cytoplasm. They are passing down towards the archegonial bottom. $\times 480$.
Fig. 4. Proembryonal second division in the metaphase. $\times 480$.
Fig. 5. Four nucleus stage. $\times 480$.
Fig. 6. Eight nucleus stage in which wall-formation is taking place. $\times 480$.
Fig. 7. Eight nucleus stage after the wall-formation. $\times 480$.
Fig. 8. Mitosis in the upper tier. $\times 480$.
Fig. 9. Mitosis in the lowest embryonic tier. The cells of prosuspensor do not elongate yet. $\times 480$.
Fig. 10. Meiotic metaphase in a pollen mother-cell. $\times 1550$.

PLATE XI.

- Fig. 11. Early stage of the prosuspensor elongation. $\times 90$.
Figs. 12-13. Tip of the prosuspensor in early stages. One or two cells situated at the apex are the cap-cell. $\times 190$.
Figs. 14-16. A group of the embryonic units at the tip of the prosuspensor. $\times 190$.
Fig. 17. More advanced stage of the embryonic tier. Primary suspensor is somewhat elongated. $\times 190$.
Fig. 18. Separated groups of embryonal cells at the tip of a primary suspensor. $\times 190$.
Fig. 19. Four embryos at the tip of a primary suspensor. One embryo is not visible in the figure. $\times 190$.





STUDIES ON FRESHWATER BRYOZOA OF JAPAN I

By

MAKOTO TORIUMI

Biological Institute, Tôhoku Imperial University, Sendai, Japan

(With Plates XII, XIII and 15 text-figures)

(Received November 14, 1940)

INTRODUCTION

Freshwater Bryozoa are rather common in Japan, being often found in lakes, ponds, pools, rivulets, etc. but up to the present time this group of animals has been insufficiently studied.

The first writings dealing with the freshwater Bryozoa of Japan were published in 1887 by KRAEPELIN who reported on the statoblasts of *Plumatella princeps* var. *emarginata*, the material having been obtained in Yedo (Tôkyô).

In 1891, OKA described a species named *Pectinatella gelationsa*, and gave a precise account of it based upon his observations.

In 1906, the same writer noted another species, *Pectinatella davenporti*, the specimens being taken from Kasumigaura.

In 1907, the same writer enumerated four species of freshwater Bryozoa from Japan, viz. *Pectinatella gelatinosa* OKA, *P. davenporti* OKA, *Plumatella repens* LAMARK and *P. casmiana* OKA. Of these species *P. casmiana* was described as a new species the description being based upon the specimens secured from Kasumigaura.

In 1908, the same writer erected a new genus *Stephanella* to receive *Stephanella hina*, a species named by him.

In 1911, the same writer gave a further account dealing with *Pectinatella davenporti* which had been described by him in 1906 and which is mentioned above. In this report he mentioned that the present species is synonymous with *Lophopodella carteri* (HYATT).

In 1912, the same writer obtained a species of Bryozoa from the waterworks of Okayama City and identified it as *Paludicella articulata* (EHRENBERG).

In 1918, KAWAMURA published a book named "Freshwater Biology of Japan I", and mentioned in it the existence of the following four

genera of freshwater Bryozoa in Japan, viz. *Plumatella*, *Stephanella*, *Pectinatella* and *Lophopodella*.

In the same work he also reported on the occurrence of *Fredericella sultana* (BLUMENBACH) in Lake Biwa.

In the year 1921, MIYASITA contributed an article concerning the locomotion of *Lophopodella carteri*. The material used in his experiments was collected in the vicinity of Tokyo.

In the "Monograph on Japanese Animals" (Nippon Dôbutu Zukan) published in 1927, YAITIRÔ OKADA mentioned four species of freshwater Bryozoa among those to be found in Japan, viz. *Paludicella articulata*, *Pectinatella davenporti*, *Pectinatella gelatinosa* and *Plumatella repens*.

In 1934, TAKAHASHI reported on the existence of *Lophopodella carteri* in Formosa.

In 1935, MIYAJI reported on the statoblasts of *Cristatella mucedo* CUVIER which were found in the lakes of Raitisi and Bakko in South Saghalin in June 1934.

In 1938, HÔZAWA reported on *Cristatella mucedo* which was obtained by the present writer in Anyôzi-numa near Sendai, and this is the first record we have of the living material of this kind of animal being found in Japan. In the same report he also dealt with the locomotion velocity of this animal, and mentioned that it is about 6.1 cm per day.

Thus the forms of freshwater Bryozoa recorded from time to time as occurring in Japan are 9 in all as shown in the following list.

1. *Paludicella articulata* (EHRENBERG)
2. *Fredericella sultana* (BLUMENBACH)
3. *Plumatella repens* (LINNÉ)
4. *P. princeps* var. *emarginata* (ALLMAN)
5. *P. casmiana* OKA
6. *Stephanella hina* OKA
7. *Pectinatella gelatinosa* OKA
8. *Lophopodella carteri* (HYATT)
9. *Cristatella mucedo* Cuvier

The present writer tried to collect freshwater Bryozoa in the Kantô and Tôhoku districts of Japan during the years 1938 and 1939, and was able to obtain 13 forms representing 8 species and 5 varieties as shown in the following list.

Of these forms, 6 species and 3 variety are identical with those previously known while the remaining 2 species and 2 varieties are described for the first time.

1. *Paludicella articulata* (EHRENBERG)
2. *Fredericella sultana* (BLUMENBACH)
3. *Plumatella repens* var. *typica* (LINNÉ)
4. *P. repens* var. *emarginata* (ALLMAN)
5. *P. repens* var. *fruticosa* (ALLMAN)
6. *P. repens* var. *minuta*, n. var.
7. *P. repens* var. *casmiana* (OKA)
8. *Hyalinella punctata* (HANCOCK)
9. *H. toanensis* HÔZAWA & TORIUMI
10. *Stephanella hina* OKA
11. *Pectinatella gelatinosa* OKA
12. *Lophopodella carteri* (HYATT)
13. *Cristatella mucedo* CUVIER

Before proceeding further, the writer should like to express his hearty thanks to Professor SANJI HÔZAWA who has kindly helped the writer in many ways during the course of present study.

Acknowledgements are also due to Doctor MARY DORA ROGICK for her kindness and generosity expressed in sending to the present writer her own valuable publications and some specimens of American Bryozoa that she possessed.

Thanks are also due to Mr. NOBUO SASAKI for his kindness and generosity in giving to the writer many valuable specimens he had obtained.

The writer thanks Mr. SENJI TANITA and to Mr. MUTSUO KATÔ for their helpful advice and criticisms.

DESCRIPTION OF FORMS

Order *Gymnolaemata*

Suborder *Ctenostomata*

1) *Paludicella articulata* (EHRENBERG)

Alcyonella articulata, EHRENBERG, 1831.

Paludicella Ehrenbergi, ALLMAN, 1856, pp. 113-115, Pl. X, figs. 1-5; VANGEL, 1894, p. 153; DAVENPORT, 1904, p. 215, Pl. VI, fig. 3; HARTMEYER, 1909, pp. 57-58, Figs. 128-129; KAWAMURA, 1918, p. 175.

Paludicella articulata, KRAEPELIN, 1887, pp. 98-99, Pl. IV, fig. 107; HARMER, 1915, pp. 441-447, Pl. LXII, figs. 1-10; ROUSSELET, 1916, p. 141; ROGICK, 1935, pp. 248-249; 1940, p. 194, Pl. I, fig. 4, Pl. II, fig. 5.

Zoarium. The branches forming zoarium are rather sparse, being

either recumbent or erect, and are given off at nearly right angles from the wide part of the zooecium.

The ectocyst is not encrusted, and is pale brown in colour and transparent.

Zooecia. The zooecia are claviform and elongated. The tubular squared orifice is placed at the wide part of the zooecium. The zooecia are separated from one another by complete septa.

Polypides. The lophophore is circular in form and is provided with 16-18 tentacles. The epistome and the calyx are absent.

Hibernacula. This species produces hibernacula which correspond to the fixed statoblasts seen in the case of *Phylactolaemata*. It varies in shape but, in general, they are short spindle or conical in form.

Distribution. It occurs widely in North America, Europe, Asia and New Zealand.

In the Kantô and Tôhoku districts of Japan, it is obtained in Kogaranuma, Aomori Prefecture and in Kasumigaura near Tôkyô.

Remarks. There are not many specimens of this species in the writer's collection.

Order *Phylactolaemata*

Family *Plumatellidae*

Subfamily *Fredericellinae*

2) *Fredericella sultana* (BLUMENBACH)

Tubularia sultana, BLUMENBACH, 1779.

Fredericella sultana, HANCOCK, 1850, p. 173; ALLMAN, 1856, pp. 110-111, Pl. IX, figs. 1-7; KRAEPELIN, 1887, pp. 103-104, Pl. VII, fig. 138; VANGEL, 1894, p. 153; DAVENPORT, 1904, p. 216; ROUSSELET, 1907, p. 251; 1916, p. 141; HARTMEYER, 1909, p. 55; Figs. 121-122; ANNANDALE, 1910, p. 39; HARMER, 1915, pp. 448-449; KAWAMURA, 1918, p. 176; ROGICK, 1935, p. 250, Pl. XL, fig. 2; 1937, pp. 101-102, Fig. 1; 1940, p. 195, Pl. III, fig. 13.

Zoarium. The zoarium branches in an antler-like manner being either recumbent or rising from the substratum. The branches are rather widely spreading.

The ectocyst is sandy or grey in colour and is usually encrusted.

Zooecia. The zooecia are long, slender and nearly cylindrical. In the old part of the zoarium the zooecia are sometimes strongly keeled. The septum which is pale brown in colour is rarely present at the base of the branch.

Polypides. The lophophore is circular in form and bears from 17 to

23 tentacles.

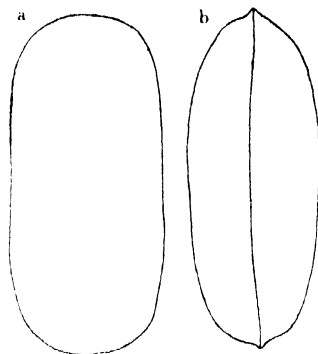
Statoblasts. The statoblasts (text-fig. 1, a, b) are bean-like in shape and are yellowish-brown in colour. They are destitute of the annulus, and are sparingly produced. The unattached side of the statoblast is smooth but shows a reticulation from the structure of cell-walls.

The length of the statoblasts is from 0.42 to 0.5 mm, and the breadth is from 0.18 to 0.26 mm.

Distribution. North and South America, Europe, Asia, Africa, Australia and New Zealand.

In the Tōhoku district of Japan, it was obtained from Kogara-numa, Aomori Prefecture and also from some small ponds near Sendai, as well as in other places.

Remarks. The Kogara-numa specimen bears an appearance different from those shown by the specimens obtained from other localities in possessing more slender branches set very closely. They are entirely recumbent and run parallel to one another.



Text-fig. 1. Fixed statoblasts of *Fredericella sultana*. a, dorsal view; b, side view ($\times 100$).

Subfamily *Plumatellinae*

3) *Plumatella repens* var. *typica* (LINNÉ)

Tubipora repens, LINNÉ, 1785.

Plumatella repens, ALLMAN, 1856, pp. 93-99, Pl. V, figs. 1-8; ZYKOFF, 1890, p. 444; VANGEL, 1894, p. 154; HARTMEYER, 1909, p. 54, figs. 118, 119; ANNANDALE, 1910, pp. 43-44; HARMER, 1913, p. 450, Pl. LXIII, fig. 21.

Plumatella polymorpha var. *repens*, KAEPELIN, 1887, p. 123, Pl. IV, fig. 119, 122, Pl. VII, fig. 139; DAVENPORT, 1904, p. 217, Pl. V, fig. 6.

Plumatella repens Phase *alpha*, *beta*, ROGICK, 1935, pp. 252-253; 1937, p. 100.

Plumatella repens var. *typica*, phase *beta*, ROGICK, 1940, pp. 201-203, Pl. III, figs. 14, 15, Pl. IV, figs. 16-19, Pl. V, figs. 20-24.

Zoarium. The zoarium is entirely recumbent, and branches in an antler-like manner.

The ectocyst is not stiff, and is colourless though occasionally it is pale yellowish brown. Rarely, it is slightly encrusted.

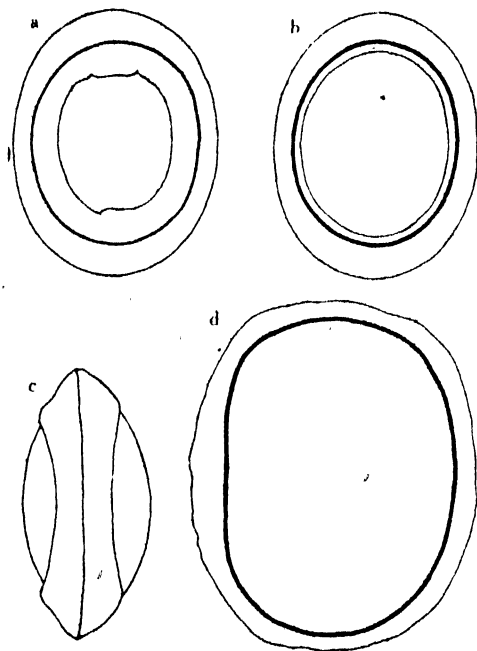
Zooecia. The zooecia are entirely recumbent, occasionally the distal part is bent upwards. When the ectocyst is encrusted the zooecium becomes

obscurely keeled. The septum is absent.

Polypides. The number of the tentacles varies from 39 to 56.

Statoblasts. The free statoblasts (text-fig. 2, a-c) are oval or nearly circular. The annulus encroaches on the capsule a little more on one surface (so-called dorsal side) than the other. The capsule is minutely mammillated.

The length of the free statoblasts is between 0.34 and 0.38 mm and the breadth is from 0.25 to 0.27 mm. The capsule varies from 0.26 to 0.28 mm in length and from 0.21 to 0.23 mm in breadth.



Text-fig. 2. Statoblasts of *P. repens* var *typica*. a, dorsal; b, ventral; c, side view of the free statoblast. d, dorsal view of the fixed statoblast ($\times 100$).

A small number of fixed statoblasts (text-fig. 2, d) were found in the specimen collected in Sendai in 1940. These are oval or elliptical in shape, possessing chitinous lamella which shows obscurely an irregular reticulation due to the vestigial air-cells. The whole length of the fixed statoblasts is about 0.46 mm long and 0.39 mm wide. The capsule is brown in colour, and is minutely mammillated being about 0.41 mm in length and 0.33 mm in breadth.

Distribution. Europe, North America, India and Japan.

This species was found in some small ponds near Sendai.

Remarks. The specimens were not obtained very abundantly, the free statoblast, however, were found at various places.

4) *Plumatella repens* var. *emarginata* (ALLMAN)

Plumatella emarginata, ALLMAN, 1844; 1856, p. 104, Pl. VII, figs. 5-10; ANNANDALE, 1907, p. 177; 1910, p. 140; HARTMEYER, 1909, p. 53, Fig. 117; HARMER,

1913, p. 453; VORSTMAN, 1928, pp. 4-5, Figs. 1, 2, Pl. I, figs. 1-4; HASTINGS, 1929, p. 130; 1929, p. 307.

Plumatella princeps var. *emarginata*, KRAEPELIN, 1887, p. 120, Pl. IV, fig. 108, Pl. V, fig. 123; DAVENPORT, 1904, p. 217, Pl. VI, fig. 5.

Plumatella emarginata forma *typica*, LEE, 1936, pp. 401-403, Fig. II.

Plumatella repens var. *emarginata*, VANGEL, 1894, p. 154; ROGICK, 1935, pp. 255-256, Pl. XLI, fig. 6; 1937, pp. 100-101; 1940, p. 198, Pl. I, figs. 1-3, Pl. III, figs. 11, 12.

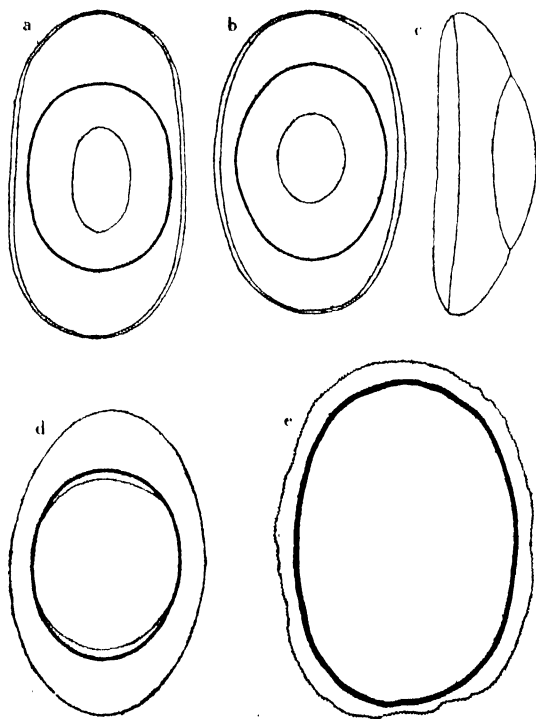
Zoarium. The zoarium is recumbent, and the branches are antler-like in form. The mode of branching is usually open, but when the zoarium grows luxuriantly, the branches are set closely giving the zoarium a rugged appearance. Sometimes the zoarium sends off several short free branches. The ectocyst is encrusted and ranges in colour from dark brown (nearly black) to sandy colour.

Zooecia. The distal part of each zooecium is never strongly bent upwards when the base is recumbent. The zooecia are long, nearly cylindrical, keeled and furrowed, the furrow being continuous with the notched margin of the orifice of the tube.

This notched part is membranous and transparent. In the vertical branches the keel as a rule disappears. Some incomplete septa in the form of a crescent are attached to the upper wall of the zoarium. Rarely, the setum is seen at the end of each zooecium. The septa are deep brown or black in colour.

Polypides. The number of the tentacles is from 30 to 50.

Statoblasts. The free statoblasts (text-fig. 3, a-d)



Text-fig. 3. Statoblasts of *P. repens* var. *emarginata*. a, b dorsal; c, side; d, ventral view of the free statoblast. e, fixed statoblast ($\times 100$).

are elongate, being truncated or subtruncated at both ends. The annulus covers the greater part of one surface, and a small part of the other surface of the capsule. Usually, the annulus is distinctly narrower on the sides than at the extremities. The capsule is brown in colour and is minutely mammillated. The length of the free statoblasts ranges from 0.36 to 0.44 mm, and the breadth is from 0.22 to 0.29 mm. The capsule is from 0.23 to 0.29 mm in length, and the breadth is from 0.17 to 0.21 mm. The fixed statoblasts are produced in great numbers. They are not always found together with the free statoblasts in the same zoarium. The fixed statoblast (textfig. 3, e) is surrounded by chitinous lamella that is minutely serrated on the margin and has no air-cells. Sometimes, however, this lamella shows an obscure and incomplete reticulation from the structure of vestigial aircells. The dorsal surface of the capsule is brown (nearly black) in colour and is minutely mammillated. The fixed statoblasts vary in length from 0.4 to 0.5 mm and in breadth from 0.23 to 0.32 mm.

Distribution. North America, Europe, Africa and Asia.

This variety is plentifully distributed all over Japan, the writer having found specimens in almost every freshwater pond, pool and lake which he has examined.

Remarks. The specimens vary in colour, in shape, in the size of the statoblasts, in the number of the tentacles and in the mode of the growth of the zoarium.

5) *Plumatella repens* var. *fruticosa* (ALLMAN)

Plumatella fruticosa, ALLMAN, 1844; 1856, p. 102, Pl. VI, figs. 3-5; ANNANDALE, 1910, p. 45; HARMER, 1913, p. 453; ROUSSELET, 1916, p. 141.

Plumatella lucifuga, JULLIEN, 1885.

Plumatella princeps var. *fruticosa*, KRAEPPELIN, 1887, p. 120, Pl. VII, fig. 148; DAVENPORT, 1904, p. 217.

Plumatella repens var. *fruticosa*, ROGICK, 1935, p. 255.

Zoarium. The zoarium branches in an antler-like manner and is attached to the substratum by means of its basal part, while most of the growth of the branches has a shrubby appearance. The ectocyst is encrusted and is yellowish-grey in colour.

Zooecia. The zooecia are slender, cylindrical, and are feebly keeled.

In well-developed zoarium, some zooecia are arranged parallel to one another being attached to one side of the branches. When the zoarium becomes old the branches will shed off the zooecia, thus leaving a ser-

ration on the branches (text-fig. 4)

Polypides. The number of the tentacles is between 32 and 50. In the healthy polypides the tentacles are golden yellow in colour.

Statoblasts. The free statoblasts (text-fig. 5, a-c) are elongated.

The annulus is as a rule much broader at the ends than on the sides, and covers the capsule as in the case of *P. repens* var. *emarginata*.

The length of the free statoblasts ranges from 0.43 to 0.51 mm and the breadth is from 0.18 to 0.23 mm. The length of the capsule is measured from 0.29 to 0.33 mm and the breadth is from 0.15 to 0.17 mm.

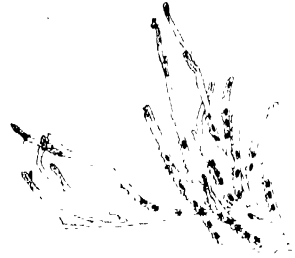
The fixed statoblasts (textfig. 5, d) are elongated in form and black in colour.

They bear serrated chitinous lamelle forming a vestigial annulus which is provided with an irregular reticulation from the cell-walls. On the dorsal surface of the sapsule a number of tubercules are acattered.

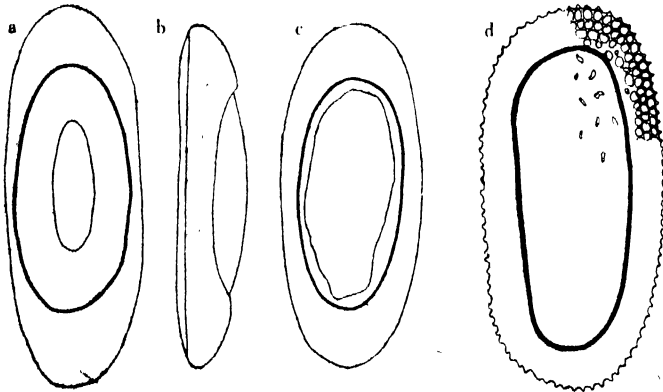
The length of the fixed statoblasts varies from 0.42 to 0.53 mm, and the breadth is from 0.18 to 0.25 mm.

Distribution. North America, Europe and Asia.

In Japan, it is obtained in some small ponds near Sendai. and in a



Text-fig. 4. A portion of an old zoarium of *P. repens* var. *fruticosa*, showing the serration on the branches due to the zoecia shed off.



Text-fig. 5. Statoblasts of *P. repens* var. *fruticosa*. a-c, free statoblasts. d, fixed statoblast ($\times 100$).

small pond near Lake Tazawa, Akita Prefecture.

Remarks. This is the first report on the occurrence of the variety in Japan.

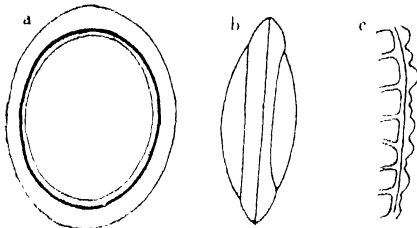
In the writer's collection, the zoaria of this variety are not very abundant, but the statoblasts were secured from various districts.

6) *Plumatella repens* var. *minuta*, n. var.

Zoarium. The zoarium is entirely recumbent, the branches being antler-like in form, and bears a resemblance to those of *Hyalinella punctata* and *P. repens* var. *typica*. It never forms a uniform flat layer and the zooecia are more slender than those of *H. punctata*.

The ectocyst is slightly swollen, soft, colourless, hyaline and is rarely encrusted.

In the case of encrusted zoarium the keel is present, and the ectocyst is not swollen and is of a sandy colour.



Text-fig. 6. Statoblasts of *P. repens* var. *minuta*. a, dorsal or ventral view; b, side view c, serration on the margin of annulus ($\times 100$).

Zooecia. The septum is not observable at any part of the zoarium.

Polypides. The tentacles range from 26 to 33 (usually 30) in number.

Statoblasts. The free statoblasts (text-fig. 6, a, b) are oval or nearly circular.

The annulus is of a dull yellow colour covering both surfaces of the capsule in a slight degree. The margin of the annulus bears irregular minute processes arranged in one row (text-fig. 6, c) as in the case of *H. punctata* and they are visible only when strongly magnified. The capsule is brown in colour and is provided with a number of scattered small nipple-shaped protuberances. The length of the statoblasts is from 0.27 to 0.3 mm. The capsule is 0.21–0.24 mm in length and is 0.17–0.19 mm in breadth. The fixed statoblasts is absent.

Distribution. This new variety is found in Tôkyô; in some small ponds near Sendai; in Tega-numa near Tôkyô and in Hatiman-numa, Namegawa-mati, Tiba Prefecture.

Remarks. This variety is not abundantly represented in the writer's

collection.

It bears a resemblance to *H. punctata*, but it may be distinguished from the latter by the following characteristics: a) The statoblasts are much smaller than those of *H. punctata*. b) The ectocyst is not strongly swollen and the zoarium is sometimes encrusted and is feebly keeled. c) The zoarium never forms a uniformly flat layer.

7) *Plumatella repens* var. *casmiana* (OKA)

Plumatella casmiana, OKA, 1907, pp. 121-123, Fig. 3; VORSTMAN, 1928, pp. 7-8, Fig. 5, Pl. I, fig. 8.

Zoarium. The zoarium is entirely recumbent and branches densely. The branches are short and lie close to each other giving a compact appearance to the zoarium.

The young zoarium appears flabelliform, but is seldom geminated.

The ectocyst is encrusted and ranges in colour from yellowish-brown to sandy colour being either opaque or semiopaque. Sometimes, near the distal part of the zooecium, the ectocyst is pigmented with a dark grey colour.

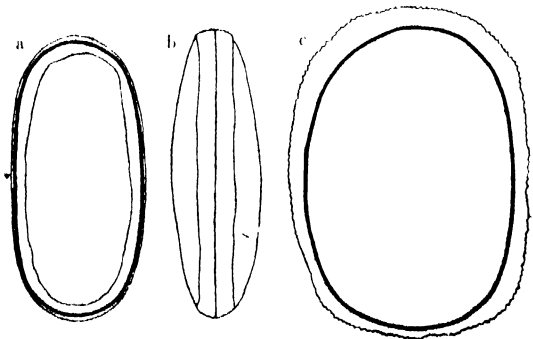
Zooecia. The zooecia are entirely recumbent, and thus the distal part of each zooecium is never bent upwards. The keel is present. Of the transverse septa, Vorstman (1928) reported that they are numerous in number, but in the Japanese specimen they are not observed at all.

Polypides. The number of the tentacles varies from 23 to 38.

Statoblasts. The free as well as the fixed statoblasts are always produced.

The free statoblasts (text-fig. 7, a, b) are very long in proportion to the breadth, being in length 0.34-0.36 mm and in breadth 0.16-0.17 mm. They are a pale yellow hue and almost colourless. The annulus is narrow and is of uniform breadth, encroaching on to the capsule on both surfaces.

All the air-cells are attached closely to the capsule. The capsule it-



Text-fig. 7. Statoblasts of *P. repens* var. *casmiana*.
a, b, free statoblasts; c, fixed statoblast ($\times 100$).

self is transparent, and is covered with scattered minute tubercles.

The fixed statoblast (text-fig. 7, c) is surrounded by a serrated chitinous lamella which shows an incomplete reticulation due to the vestigial air-cells. The capsule is brown in colour and is minutely mammillated.

The length of the fixed statoblasts is 0.36–0.43 mm and the breadth is 0.23–0.3 mm.

Distribution. Japan and Java.

In the Tōhoku district of Japan it was found at two localities. One in a small pond near Sendai; and the other in a small pond at Gosyogawara-mati, Aomori Prefecture.

In the Kantō district, it was obtained from a number of small ponds in Tōkyō; and from a small pond at Uruga-mati, Kanagawa Prefecture.

Remarks. In the writer's collection this variety is not abundantly represented.

8) *Hyalinella punctata* (HANCOCK)

Plumatella punctata, HANCOCK, 1850, p. 200, Pl. III, fig. 1; ALLMAN, 1856, pp. 100–102, Fig. 15; HARTMEYER, 1909, p. 59, Fig. 116; ANNANDALE, 1910, p. 52; 1919, p. 94; ROUSSELET, 1916, p. 141.

Plumatella vesicularis, JULLIEN, 1885; VANGEL, 1894, p. 155.

Hyalinella punctata, HASTINGS, 1929, p. 303; ROGICK, 1935, p. 251; 1940, pp. 196–198, Pl. II, figs. 6–10, Pl. V, fig. 25.

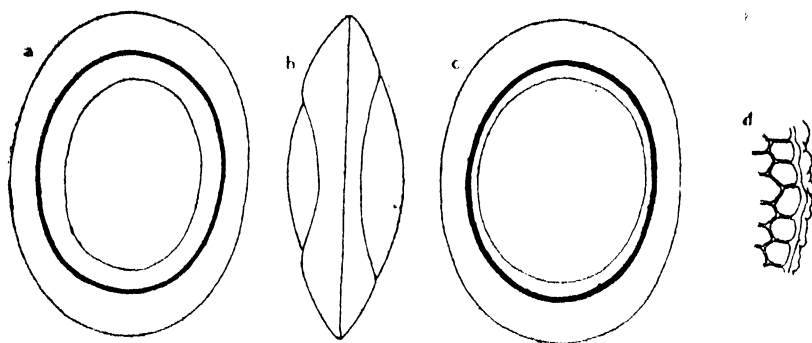
Zoarium. The zoarium is transparent, colourless and is entirely recumbent.

Usually it branches irregularly, but when it grows luxuriantly around the slender stem or narrow leaf of some water-plants, it forms rarely a hyaline mass as in the case of *Hyalinella toanensis*. It differs from the latter species, the mass being smaller and the jelly not very copious. The ectocyst is soft, swollen and hyaline.

Zooecia. Lacking the septa, the zooecia form one common coenoecium. The tips of the zooecia are conical or rounded and are lacking white spots. The keel and the furrow are absent.

Polypides. The tentacles vary from 40 to 58 in number, and the calyx of the lophophore is distinctly festooned.

Statoblasts. Free statoblasts (text-fig. 8, a–c) only are produced, and they are broad, and are nearly circular. The margin of the annulus is irregularly serrated (text-fig. 8, d) and is observable when strongly magnified. The annulus covers both surfaces of the capsule but leaving a large area in the centre. The capsule mammillated and is of brown colour.



Text-fig. 8. Statoblasts of *H. punctata*. a, dorsal; b, side; c, ventral view of the statoblasts. d, serration on the margin of annulus ($\times 100$).

The whole length of the statoblast is from 0.4 to 0.46 mm, and the breadth is 0.27–0.33 mm. The capsule is 0.29–0.33 mm long, and is 0.2–0.26 mm broad.

Larvae. The ciliated larvae were found of this species. They are about 0.45–0.6 mm long and 0.31–0.37 mm broad.

Distribution. North America, South America, Europe and Asia.

In the Tôhoku district of Japan, this species was found in some small ponds near Sendai and in Kase-numa at Siogama-mati near Sendai. In the Kantô district it was secured from Koaidame in Tôkyô.

Remarks. This is the first report on the occurrence of this species in Japan. The species seems to be widely distributed in Japan, as the statoblasts were found at various places.

9) *Hyalinella toanensis* HÔZAWA & TORIUMI

Hyalinella toanensis, HÔZAWA & TORIUMI, 1940, p. 431, Fig. 6, Pl. fig. 4.

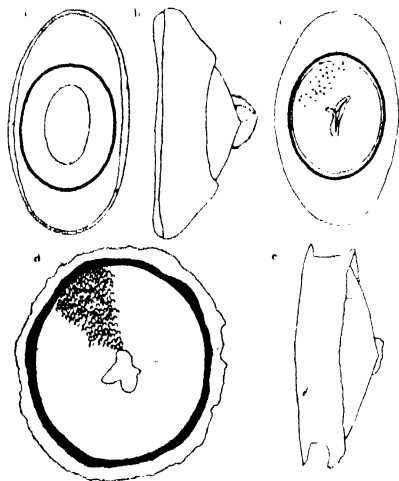
Zoarium. The zoarium forms a nodular mass growing around the stems of some water-plants.

The surface is generally not smooth owing to the conical or round tips of the zooecia projecting from it. The jelly (ectocyst) is copious, and sometimes attains to a thickness of nearly 1 cm. It is colourless and hyaline, but when preserved in spirit it becomes somewhat hard and elastic. Of the mode of branching the zoarium shows some characteristic features similar to those seen in the case of *Hyalinella indica* ANNANDALE.

Zooecia. The zooecium does not represent the form resembling figure L as is the case of *H. indica*. It is either straight or slightly bent upwards, its tip projecting from the coenoecial jelly.

Polypides. The number of the tentacles varies from 50 to 73. The calyx is distinctly festooned.

Statoblasts. The free statoblasts (text-fig. 9, a-c) are elongated being somewhat rhombic, and are rounded at both ends. The annulus encroaches a little more on one surface (so-called dorsal side) than the other, and is narrower on the lateral sides than at the ends. The annulus does not curve as in the case of *H. indica*. The capsule has a blunt process in the centre of one surface (so-called ventral side), and shows a reticulation of cellwalls on both surfaces. The large blunt process is provided with a peculiar formed appendage which is spinous and transparent (text-fig. 9, d, e). They may be easily detached from the process.



Text-fig. 9. Statoblasts of *H. toanensis*. a, dorsal; b, side; c, ventral view of the free statoblasts d, dorsal; e, side view of the fixed statoblast ($\times 50$).

The length of free statoblasts ranges from 0.5 to 0.6 mm, and the breadth is from 0.3 to 0.37 mm. The capsule is 0.31–0.39 mm long, while the breadth is 0.24–0.3 mm. The fixed statoblasts (text-fig. 9, d, e) are rarely produced. It is generally oval, but rarely circular in form, and is almost black in colour. It is provided with a serrated, stout chitinous lamella of a brown or black colour.

The capsule possesses one large process in the centre of the dorsal surface. Sometimes it is covered with scattered black tubercles. The blunt process of the fixed statoblast is provided with an irregularly formed

appendage as in the case of free statoblast. The appendage of fixed statoblast is not spinous. The fixed statoblasts are adhering to the inner side of the zooecia. The length of the fixed statoblasts is from 0.52 to 0.59 mm, and the breadth is from 0.49 to 0.5 mm.

Distribution. Japan and Manchoukuo.

In the Tôhoku district of Japan, it was also secured from the following localities, viz. two small ponds near Sendai; a small pond near Nimai-basi Station, Iwate Pref.; Yabudai-no-ike, Akita Pref.; Nanko near Sirakawa Station, Hukusima Pref.; Nasi-ike near Kagamiisi Station, Huku-

sima Prefecture.

Remarks. This species bears a close resemblance to *H. indica* ANANDALE, differing only in the characteristics of the statoblast. The zoarium forms usually a small mass, of about 1 cm wide, from 2 to 3 cm or more long and 1 cm high. This species was first found at Toan in Manchoukuo by KAWAMURA and MIYAJI in 1938, and was described by HÔZAWA and the present writer.

10) *Stephanella hina* OKA

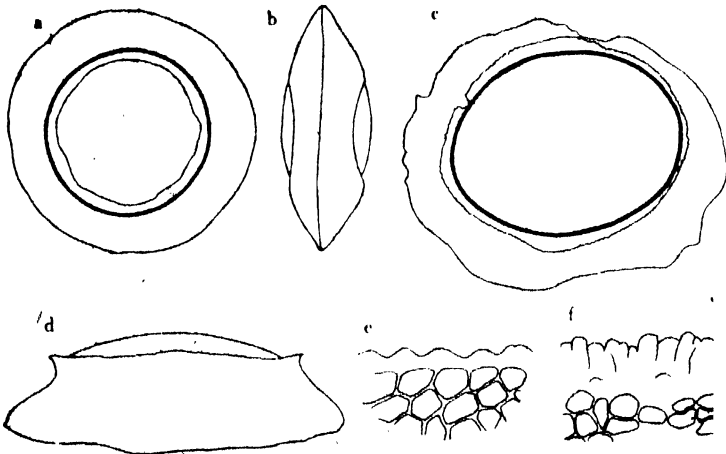
Stephanella hina, OKA, 1908, pp. 277-285, Pl. X, figs. 1-5; ROUSSELET, 1916, p. 141.

Zoarium. The zoarium forms an almost flat gelatinous mass covering the substratum. It bears a resemblance to the members of *Hyalinella* except for the jelly which is very soft. The surface is rather smooth. The jelly is copious, colourless and transparent.

Zooecia. The zooecia are cylindrical and are embedded in the jelly. These are connected by means of a slender ramifying stolon creeping over the substratum.

Polypides. The epistome is present. The lophophore has very short arms and bears 36-40 tentacles.

Statoblasts. The free statoblast (text-fig. 10, a, b) is circular, possessing neither spines nor a serration on the margin of the annulus. The free statoblasts are 0.3-0.34 mm in diameter, and the capsules are from 0.21



Text-fig. 10. Statoblasts of *Stephanella hina*. a, b, free statoblasts; c, d, fixed statoblasts; d, side view of the same; e, f, serration of the chitinous lamella ($\times 100$).

to 0.23 mm in the same. The fixed statoblast (text-fig. 10, c, d) is an oval nearly approaching a circle in form. The capsule is pale yellowish-brown in colour and is transparent, and shows a reticulation from cell-walls. The chitinous lamella is present. It is narrow and serrated. The length of the fixed statoblasts is between 0.41 and 0.46 mm, and their breadth is between 0.32 and 0.34 mm. The capsule is from 0.35 to 0.42 mm in length and is from 0.28 to 0.31 mm in breadth.

Distribution. The distribution of this species is limited in Japan.

Remarks. Of the present species only one specimens was obtained from the Tôhoku district of Japan. It was an old zoarium bearing some degenerating polypides and producing numerous statoblasts. The free statoblasts were found almost in every pond.

Family Lophopodidae

11) *Pectinatella gelatinosa* OKA

Pectinatella gelatinosa, OKA, 1890; 1907, p. 716; ANNANDALE, 1907, p. 148; 1910, p. 56; Rousselet, 1916, p. 141; Hastings, 1929, p. 305.

Pectinatella burmanica, ANNANDALE, 1910, p. 56; ROUSSELET, 1916, p. 141; Vorstman, 1928, p. 12, Fig. 9, Pl. I, fig. 12; Hastings, 1929, p. 305.

Zoarium. The zoarium is spherical in form and possesses the coenoecial jelly around the polypides. Usually it is about 2 cm in diameter, but in the specimen preserved in spirit they are considerably shrunken. In some well-developed compound zoaria the jelly is very copious attaining to a thickness of 1.5 cm. The jelly is colourless, transparent and soft.

Zooecia. The zooecia are embedded in the coenoecial jelly radiating from the centre of the zoarium.

Polypides. The polypides are larger than those of the members of the genus *Plumatella*.

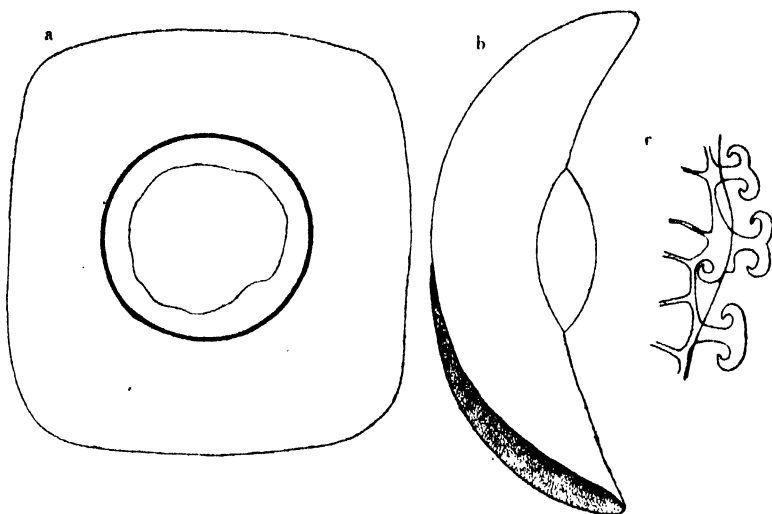
The number of the tentacles ranges from 72 to 101.

Statoblasts. Only free statoblasts (text-fig. 11, a, b) are produced. They are nearly circular, and are dark brown in colour. The surface of the statoblast is curved like that of a saddle. The annulus is large in proportion to the capsule and covers the latter slightly on both surfaces.

Numerous processes (text-fig. 11, c) are present surrounding the annulus, each bearing at its extremity a pair of hooks.

Distribution. Japan, India and Java.

The present writer was able to obtain this species from the following



Text-fig. 11. Statoblasts of *Pectinatella gelatinosa*. a, dorsal or ventral view; b, side view ($\times 50$). c, paired hooks of annulus ($\times 450$).

two localities: -- Usiku-numa near Tôkyô; Koaidame in Tôkyô.

Remarks. This species is very common in the Kantô district of Japan, the writer, however, was unable to find it in the Tôhoku district.

12) *Lophopodella carteri* (HYATT)

Lophopus sp., CARTER, 1859, p. 355, Pl. VIII, figs. 8-15.

Pectinatella carteri, HYATT, 1865; JULLIEN, 1885.

Pectinatella davenporti, OKA, 1907, pp. 117-120, Figs. 1, 2; 1907, p. 716; ANNANDALE, 1907, p. 148; p. 268; ROUSSELET, 1916, p. 141.

Lophopodella carteri, ANNANDALE, 1919, p. 97; TAKAHASHI, 1934, pp. 347-350; LEE, 1936, pp. 399-401, Fig. 1.

Lophopodella carteri davenporti, ANNANDALE, 1919, p. 97.

Lophopodella carteri var. *davenporti*, HASTINGS, 1929, p. 305; ROGICK, 1934, pp. 420-421.

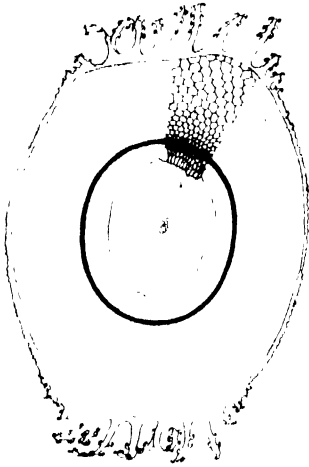
Lophopodella carteri var. *typica*, ROGICK, 1934, pp. 416-424, Pl. XLI, figs. 1-3, Pl. XLII, fig. 1; 1935, p. 250.

Zoarium. The zoarium is small, transparent, sacciform or lobate, consisting of 30-40 polypides, and is about 0.5-1 cm in length and breadth. A delicate transparent ectocyst surrounds the zoarium. The number of lobes varies from 3 to 5 and they may change in form owing to the elasticity of the body-wall.

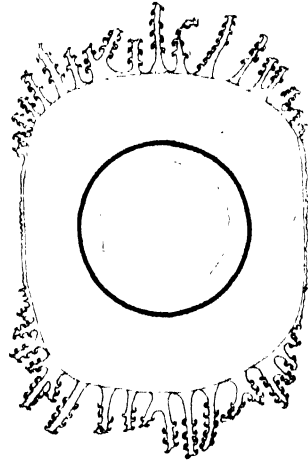
Zooecia. The zooecia are retracted into a common cavity, and there exist no partitions within the coenocodium.

Polypides. The polypides are large in proportion to the zoarium. The number of the tentacles is from 74 to 83.

Statoblasts. Only the free statoblasts (text-fig. 12) are seen. They are flat, sometimes curved in saddle-like manner, more or less elliptical in shape, and are rounded or subtruncated at the ends. The annulus is large in proportion to the capsule, and encroaches a little on both sur-



Text-fig. 12. Statoblast of *Lophopodella carteri* ($\times 50$).



Text-fig. 13. One statoblast of *Lophopodella carteri* bearing 19 spines at each extremity. The most frequent number of the spines is 8 or 11 at each extremity as shown in text-fig. 12. This statoblast was secured at Izu-numa near Sendai by S. HÔZAWA on IX-14-1931. ($\times 50$).

faces of the capsule. The length of the statoblasts including the spines varies from 1.2 to 1.3 mm, and the breadth is from 0.75 to 0.8 mm.

The length of the capsule is about 0.55 mm and the breadth is 0.45–0.55 mm.

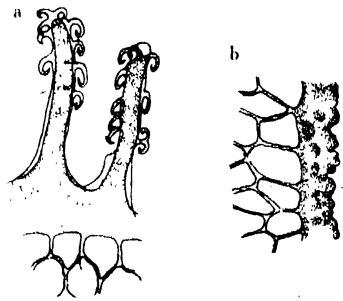
The number of the spines is between 6 and 16, usually being from 8 to 11. OKA (1907) described statoblasts bearing from 18 to 20 of spines at each end, but such specimens were not found among those obtained by myself in the Kantô and Tôhoku districts of Japan. Each of these spines bears on each side 3–11 (usually 7–8) small, semicircularly curved, flat, generally sharply but sometimes bluntly terminated barbs (text-fig. 14, a). On each side (excepting the extremities) of the annulus there may be seen an irregular minute serration (text-fig. 14, b).

Distribution. North America, Africa, India, Java, China and Japan.

In the Tōhoku district of Japan, this species was found in the following localities:—Three small ponds near Sendai; Kase-numa at Siogama-mati near Sendai; Kogara-numa, Aomori Pref.; Ebita-numa near Nakamura Station, Hukusima Prefecture. In the Kantō district it was obtained from a small pond at Hisazumi-mura, Tiba Prefecture.

Remarks. The writer was not able to detect any difference between *L. carteri* var. *typica* and var. *davenporti*.

The zoarium resembles the young zoarium of *Pectinatella gelatinosa* both in general structure and in microscopical structure, but it may be distinguished from the latter by the smaller size of polypides.



Text-fig. 14. a, two spines taken from one extremity of statoblast of *Lophopodella carteri* ($\times 300$). b, serration seen on the lateral margin of annulus ($\times 300$).

Family *Cristatellidae*

13) *Cristatella mucedo* CUVIER

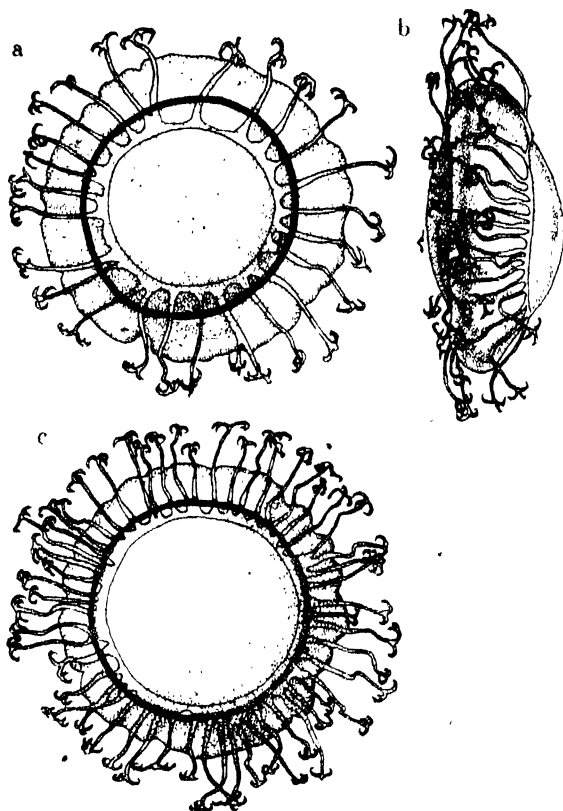
Cristatella mucedo, CUVIER, 1798; ALLMAN, 1856, pp. 77–80, Pl. I, figs. 1–8; ZYKOFF, 1890, p. 444; VANGÉL, 1894, p. 155; HARTMEYER, 1909, p. 56, figs. 124–115; ROUSSELET, 1916, p. 141; HÖZAWA, 1939 p. 104.

Zoarium. The zoarium is worm-like being from 2 to 20 cm long, and is about 0.5 cm broad. The polypides are embedded in the coenoecium and are arranged on five or six (sometimes four) concentric lines leaving a long and narrow space in the centre.

In the central space already mentioned a number of brown or yellowish brown spots due to old degenerated polypides may be seen. The polypides are formed near the margin of the zoarium.

Polypides. The number of the tentacles varies from 71 to 99.

Statoblasts. The statoblasts (text-fig. 15) are circular, being surrounded by the annulus, and bear many marginal spines. One surface of the statoblast is a little more convex than the other. The annulus which to a small extent covers the capsule on one surface is dull yellow in colour. The capsule is of brown colour (sometimes nearly black) and is mammillated. The spines are produced radially from both surfaces of the capsule. These spines are found 12–30 on one surface and 29–50 on the other.



Text-fig. 15. Statoblasts of *Cristatella mucedo*. a, dorsal view; b, side view; c, ventral view ($\times 40$)

each terminating in two, three or four curved hooks resembling grapnels.

The statoblasts measure from 0.8 to 0.9 mm or more in diameter excluding the spines.

Larvae. Ciliated larvae which are globose in form are produced. The number of the polypides of each larva is from 2 to 5 or more. They vary in size, but are usually about 1 mm in diameter.

Distribution. Europe. North America and Japan.

In the Tôhoku district of Japan this species was found in the following localities: — Some small ponds near Sendai; Kase-numa at Siogama-

mati near Sendai; Izu-numa near Sendai; Ezogate-no-tameike, Morita-mura and Kogara-numa. Aomori Prefecture.

Remarks. In 1934, SASAKI found some statoblasts of this species attached to a freshwater sponge, *Spongilla lacustris*, collected at Tisima by A. TANAKA and R. HOSINO in 1934 and sent to him for study. In the same year MIYAJI found in South Saghalin many statoblasts of the present species attached to dried water-plants. The zoarium, however, was not found at that time.

In 1938, the writer obtained a number of zoaria of this species in Anyôzi-numa, near Sendai, and of these HÔZAWA has contributed an article entitled "*Cristatella mucedo* found in Japan" to the Zoological Magazine of Japan of 1939 (p. 104).

This is the first full record on the occurrence of this species in Japan.

LITERATURE CITED

- ALLMAN, G. J., 1856. Monograph of Fresh-water Polyzoa. Ray Soc., London.
- ANNANDALE, N., 1910. Materials for a revision of the Phylactolaematous Polyzoa of India. Rec. Ind. Mus. Vol. 5, pp. 37-57.
- , 1915. The Genus *Austrolella* and some allied species of Phylactolaematous Polyzoa. Ibid. Vol. 11, pp. 163-169.
- , 1919. Sponges, Hydrozoa and Polyzoa of Seistan. Ibid. Vol. 18, pp. 83-97.
- CARTER, H. J., 1859. On the Identity in Structure and Composition of the so-called seed-like Body of *Spongilla* with the winter egg of the Bryozoa. Ann. Mag. Nat. Hist. III: 3, pp. 331-343.
- DAHLGREN, U., 1934. A species and Genus of Fresh-water Bryozoon New to North America. Science, Vol. 79, p. 510.
- DAVENPORT, C. B., 1904. Report on the Fresh-water Bryozoa of the United States. Proc. U. S. Nat. Mus. Vol. 27, pp. 211-221.
- HANCOCK, A., 1850. On the Anatomy of the Freshwater Bryozoa, with Descriptions of three species. Ann. Mag. Nat. Hist. II: 5, pp. 173-204.
- HARMER, S. F., 1913. The Polyzoa of Waterworks. Proc. Zool. Soc. London. 1913, pp. 426-457.
- HARTMEYER, R., 1909. 'Bryozoa, Moostierchen' in Brauer's "Die Süßwasserfauna Deutschlands", 19, pp. 49-58.
- HASTINGS, B. A., 1929. Phylactolaematous Polyzoa from the "Pans" of the Transvaal. Ann. Mag. Nat. Hist. X: 3, pp. 129-137.
- HÖZAWA, S., 1939. *Cristatella mucedo* Cuvier found in Japan. A report which was made on the 14th Annual Meeting of the zoological Society of Japan of 1938 and was published in the Zoological Magazine Vol. 51, No. 2, p. 104.
- JULLIEN, J., 1885. Monographie des Bryozoaires d'Eau Douce. Bull. Soc. Zool. de France, X, 119 pp.
- KAWAMURA, T., 1918. The Fresh-water Biology of Japan. (Nippon Tansui Seibutugaku). Vol. I, Ectoprocta, pp. 172-177.
- KRAEPELIN, K., 1887. Die Deutschen Süßwasser-Bryozoen. Abh. Naturwiss. Ver Hamburg.
- LEE, L., 1936. Notes on some Fresh-water Polyzoa of Peiping. Sinensia, Vol. 7, pp. 399-407.
- LEIDY, J., 1879. On *Cristatella idae*. Proc. Acad. Nat. Sci. Philadelphia. 1879, pp. 203-204.
- MIYAJI, D., 1934. On the Statoblasts of Fresh-water Polyzoa, *Cristatella*, found in South Saghalin. (Karahuto nite Hakken sitaru Tansui-Kokemusi *Cristatella* no Kyûga.) Botany and Zoology, Vol. 3, No. 5, p. 125.
- OKA, A., 1891. Observations on Fresh-water Polyzoa (*Pectinatella gelatinosa*, nov. sp.) Journ. Coll. Sci. Tokyo Imp. Univ. Vol. IV, Part I, pp. 89-150.
- , 1906. A new species of Fresh-water Bryozoa. (*Pectinatella davenporti*), Zool. Mag. Tokyo, Vol. 18, pp. 307-310.
- OKA, A., 1907. Eine dritte Art von *Pectinatella* (*Pectinatella davenporti* n. sp.). Zool. Anz. 31, pp. 716-718.
- , 1907. Zur Kenntnis der Süßwasser-Bryozoenfauna von Japan. Annot. Zool. Japan. Vol. VI, pp. 117-123.

- OKA, A., 1908. Über eine neue Gattung von Süßwasserbryozoen (*Stephanella* n. g.). Ibid. pp. 277-284.
- , 1911. Some reports on Fresh-water Bryozoa. Zool. Mag. Tokyo. Vol. 23, pp. 588-589.
- , 1912. Some reports on Fresh-water Bryozoa. Ibid. Vol. 24, p. 591.
- POTTS, E., 1884. On a supposed New Species of *Cristatella*. Proc. Acad. Nat. Sci. Philadelphia. 1884, pp. 193-199.
- RAO, H. S., 1929. Sponges and Polyzoa of the Indawgyi Lake Burma. Rec. Ind. Mus. Vol. 31, pp. 269-271.
- RIDLEY, S. O., 1890. On the Characters of the Genus *Lophopus*, with a Description of a new Species from Australia (*L. lendenfeldi*). Journ. Linn. Soc. London (Zool.), Vol. XX, pp. 61-64.
- ROGICK, M. D., 1934. Studies on Freshwater Bryozoa. I. The Occurrence of *Lophopodella carteri* (Hyatt) 1886 in North America. Trans. Amer. Micro. Soc. Vol. LIII, No. 4, pp. 416-424.
- , 1935. II. The Bryozoa of Lake Erie. Ibid. Vol. LIV, No. 3 pp. 245-263.
- , 1936. IV. On the Variation of Statoblasts of *Lophopodella carteri*. Ibid. Vol. LV, No. 3, pp. 327-333.
- , 1937. V. Some Addition to Canadian Fauna. Ohio Jour. Sci. Vol. XXXVII, No. 2, pp. 99-104.
- , 1940. IX. Additions to New York Bryozoa. Trans. Amer. Micro. Soc. Vol. LIX, No. 2, pp. 187-204.
- ROUSSELET, C. F., 1907. Zoological Results of the Third Tanganyika Expedition conducted by Dr. W. A. CUNNINGTON, 1904-1905. Proc. Zool. Soc. London, Vol. 1, pp. 250-257.
- , 1916. Statoblasts of Fresh-water Polyzoa. Journ. Roy. Micro. Soc. London, 1916, pp. 141-142.
- TAKAHASI, S., 1934. Sur *Lophopodella carteri* (Hyatt) d'eau douce, originaire de Formosa. Annot. Zool. Japanese, Vol. 14, No. 3, pp. 347-350.
- VANGEL, E., 1894. Daten zur Bryozoen-Fauna Ungarns. Zool. Anz. 17, pp. 153-155.
- VORSTMAN, A. G., 1928. Some Fresh-water Bryozoa at West Java. Treubia, Vol. 10, pp. 1-14.
- ZYKOFF, W., 1890. Zur Fauna der Süßwasser Bryozoen der Umgegend von Moskau. Zool. Anz. 1890. p. 444.

EXPLANATION OF PLATES

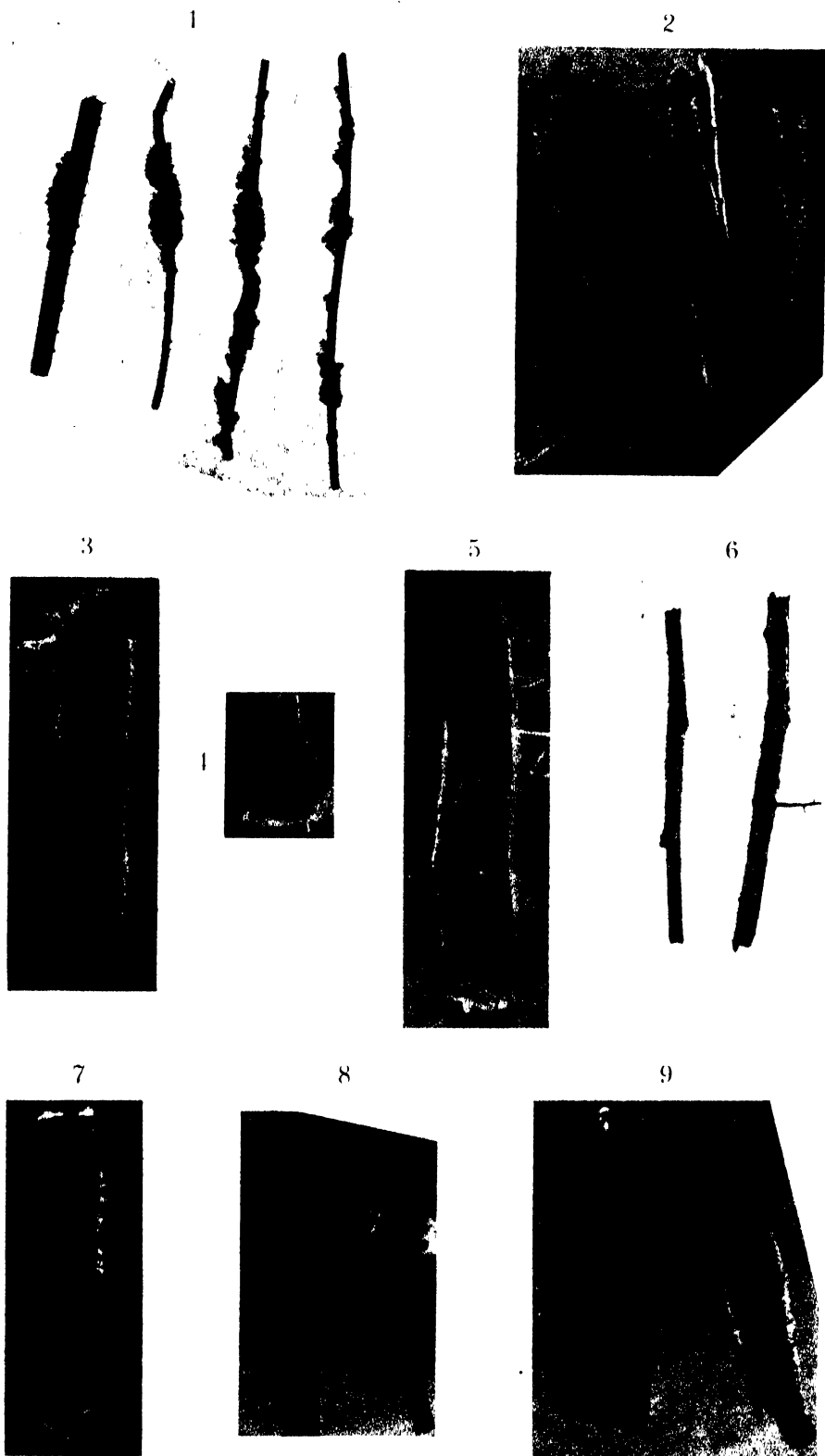
Plate XII.

- Fig. 1. *Hyalinella toanensis* HÔZAWA & TORIUMI from Gatugi-numa near Yanagita Station, Akita Prefecture. The zoaria possess some fixed statoblasts
- Fig. 2. *Hyalinella punctata* HANCOCK from a small pond in Sendai
- Fig. 3. *Plumatella repens* var. *typica* (LINNÉ) from a small pond in Sendai.
- Fig. 4. *Plumatella casmiana* OKA from a small pond in Tokyo.
- Fig. 5. *Plumatella casmiana* OKA from a small pond at Gosityogawara-mati, Aomori Prefecture.
- Fig. 6. *Plumatella repens* var. *emarginata* (ALLEN) from a small pond near Nimai-basi Station, Iwate Prefecture.
- Fig. 7. *Plumatella repens* var. *minuta*, n. var. from a small pond near Sendai.
- Fig. 8. the same
- Fig. 9. *Plumatella repens* var. *emarginata* (ALLEN) from a small pond in Sendai

Plate XIII.

- Fig. 10. *Pectinatella gelatinosa* OKA from Tega-numa near Tokyo.
- Fig. 11. *Hyalinella toanensis* HÔZAWA & TORIUMI from a small pond in Sendai.
- Fig. 12. *Hyalinella punctata* (HANCOCK) from Koaidame in Tokyo
- Fig. 13. *Lophopodella carteri* (HYATT) from a small pond near Sendai.
- Fig. 14. *Fredericella sultana* (BLUMENBACH) from Tuta-numa, Aomori Prefecture.
- Fig. 15. *Plumatella casmiana* OKA from a small pond at Gosityogawara-mati, Aomori Prefecture. Showing features somewhat different from the specimen mentioned in Pl. XII, fig. 5.
- Fig. 16. *Cristatella mucedo* CUVIER from a small pond in Sendai.
- Fig. 17. *Plumatella repens* var. *fruticosa* (ALLEN) from a small pond in Sendai.

(All natural size)



TORIUMI photo.

M. TORIUMI: Freshwater Bryozoa of Japan.

10



11



12



13



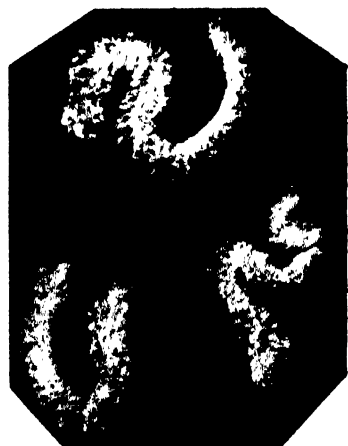
15



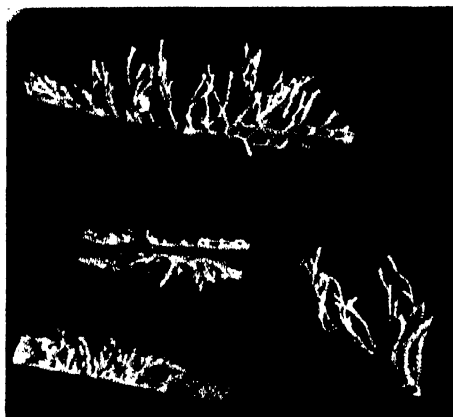
14



16



17



TORIUMI photo.

M. TORIUMI: Freshwater Bryozoa of Japan.

THE EARLY DEVELOPMENT OF CYNTHIA RORETZI

By

ETURÔ HIRAI

Biological Institute, Tôhoku Imperial University, Sendai

(With 8 text-figures)

(Received November 15, 1940)

The ascidian egg has long been studied by many authorities, as it presents many problems in connection with its development. In these problems, an extensive cytoplasmic movement, which was found by E. G. CONKLIN, is the important phenomenon in the cytoplasmic maturation. As the present writer takes great interest in this phenomenon, he carried out, in the spring of 1940, an observation of the early development of *Cynthia roretzi* DRASCHE which is abundant in Japan. He wishes to record its result in this paper.

Further, he is much indebted to Prof. Dr. E. NOMURA¹⁾, Dr. I. MOTOMURA and Mr. K. OKADA for their kind suggestions and advices. He wishes to thank to Mr. K. NARITA and the courteous officials of Sendai Railway Station, who studied his convenience in bringing the materials from Kesennuma and Onagawa, Miyagi Prefecture, to the laboratory in Sendai. At the same time he wishes also to thank to Mr. HATANAKA of the Onagawa Oceano-chemical Laboratory who supplied materials to him while he was engaged on the work.

MATERIAL AND METHOD

The observation was carried out in the laboratory of the Tôhoku Imperial University in Sendai in January, 1940. The material used in this study was supplied to the laboratory in Sendai from the culture-station of *Cynthia roretzi* DRASCHE at Hinatagai, Karakuwamura, Miyagi Prefecture, and from the Onagawa Oceano-chemical Laboratory. The materials were sent about eight times during January. Artificial fertilization was only possible in those materials which were sent during the latter part of January, from the 18th onwards.*

The colour of the ovary in the materials was brown, when the eggs

1) It is, here, worthy of note that the present investigation was carried out at the expense of Kagakukankyuhi from the Department of Education, Japan.

in the ovary were too young to fertilize, but the more the eggs approach maturation the darker brown the ovary becomes. When the ovary becomes almost black the eggs in it are in the best condition to fertilize.

Artificial insemination was carried out in the artificial sea-water of HERBST (1904) which was diluted to the salinity of the Pacific coast at Matusima following I. MOTOMURA (1935).

Cynthia roretzi is hermaphrodite, the male and the female organs adhering so closely to each other that it was impossible to divide the male from the female cells when they were removed from the gonad. When, therefore, the eggs were removed from the oviduct and placed in the sea-water, the spermatozoa also become free in the water. Self-fertilization is possible in this sea-squirt. The artificially inseminated eggs were placed in glass dishes containing the sea-water the temperature of which was kept at 14°C. in the thermostat. Observations were carried out on the living egg and on the fixed material. For the purpose of fixation, CHAMPY's mixture and ZENKER's solution was used in which there was 10% of formalin instead of acetic acid. The fixed material was sectioned 10 μ thickness, by paraffin method. After sectioning, the sections were stained by MALLORY's connective tissue staining method, CHAMPY-KULL's triple staining, HEIDENHEIN's iron-hematoxylin and DELA-FIELD's hematoxylin.

OBSERVATION

THE LIVING EGG

The primary oöcyte of *Cynthia roretzi*, which was removed from the oviduct and was placed in the artificial sea-water, is spherical in shape, and in colour yellow by reflex light, and is enclosed by the follicular epithelia, the chorion and the layer of test-cells, in a similar structure as E. HIRAI ('39) described with regard to the ovarian egg of the same species. The diameter of the living oöcyte including these surrounding envelopes is about 310 μ . and that of the oöcyte is about 270 μ .

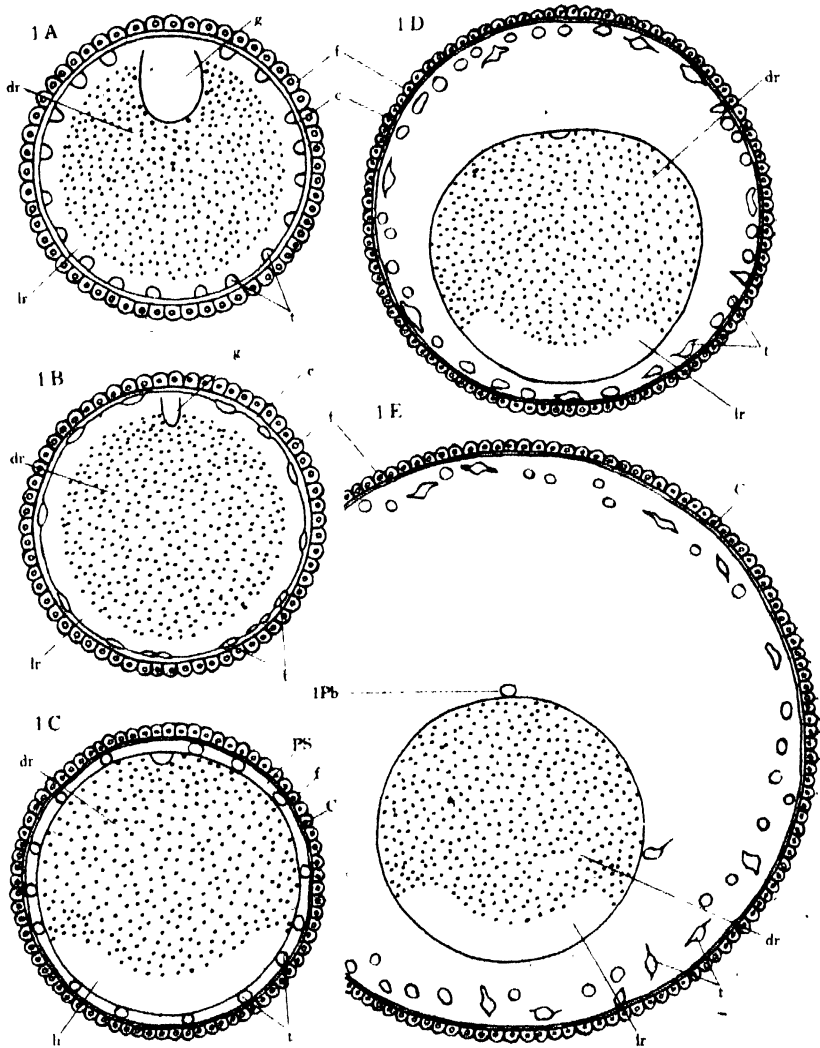
The stage at which the egg was placed in the sea-water was at the end of the growth period, when it has a large transparent germinal vesicle, the diameter of which is approximately one-third of the entire oöcyte, at one pole of the egg (Text-fig. 1 A, g). The main structural feature of the oöplasm is that it consists of two regions. One is a concentric dark region which occupies most of the central part of the oöcyte under the microscope, almost all of this region being filled with yolk

granules in mass (Text-fig. 1 A, dr). The other region is peripheral and is lighter in colour, and it surrounds the dark region with equal thickness. The latter contains small cytoplasmic granules, but no yolk (Text-fig. 1 A, lr.). The test-cells, which are embedded in the peripheral light region, are similar colour to the light region, so that they are scarcely observable in the living egg (Text-fig. 1 A, t).

About an hour after the egg is placed in the sea-water, the germinal vesicle begins to decrease in size, and after two hours it becomes merely a clear spot at a pole of the oöcyte (Text-fig. 1 B and 1 C, g). As the germinal vesicle disappears, the light region at the peripheral of the oöcyte, with the exception of the antipolar hemisphere, also disappears and at the end of two hours is replaced by dark yolk. The light region at the antipole becomes twice as wide and clearer than before (Text-fig. 1 B and 1 C, dr and lr). Whilst these changes are occurring, the test-cells are expelled from the peripheral layer into the space between the chorion and the egg, where they become spindle-shape. This space is at first indistinct, but soon a perivitelline space becomes distinct between the chorion and the egg, and the test-cells become free in this space recovering their spherical shape (Text-fig. 1 B and 1 C, t and ps). Until the appearance of the perivitelline space, it has not been possible to distinguish the egg-membrane from the chorion as E. HIRAI ('39) has already stated with regard to the ovarian egg, but when the space appears the thin egg-membrane is recognizable around the oöcyte. After the appearance of the perivitelline space the chorion with the follicular membrane swells and becomes about twice as long as the diameter of the oöcyte, and the future development of the egg progresses in this membrane (Text-fig. 1 C, 1 D and 1 E). The first polar body is formed at the pole of the egg about four and half hours after the egg is placed in the sea-water, and about twenty minutes later the second polar body is cut off (Text-fig. 1 E, 2 F and 2 G, 1 pb and 2 pb). About this stage, the light region at the antipole of the egg moves towards the pole along one side of the egg-surface (Text-fig. 2 H, lr). When the end of this region reaches to a point a little above the egg-equator the direction of the movement turns towards the centre of the egg (Text-fig. 2 J, lr). When the end of this region approaches the centre of the egg, the first cleavage occurs. It occurs about five and half hours after the egg is placed in the sea-water (Text-fig. 2 K).

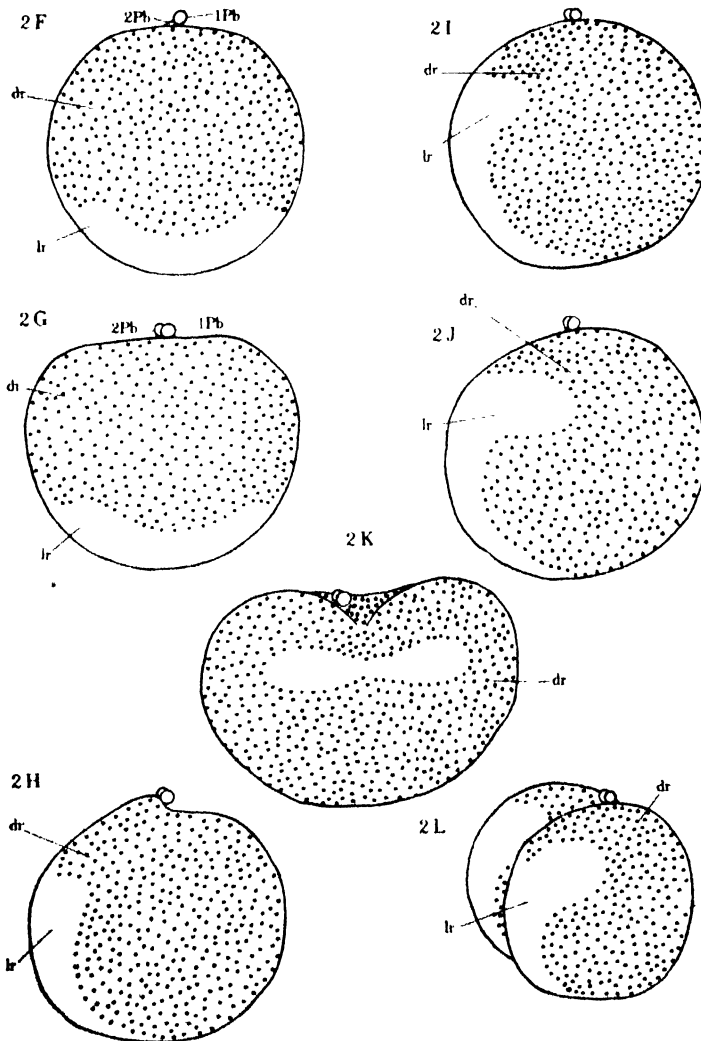
The egg of *Cynthia roretzi* DRASCHE, when removed from the oviduct, is enclosed by membranes as mentioned before. The test-cells are em-

bedded in the cortical cytoplasm of the oöcyte, so that the outline of the egg is very broken in shape (Text-fig. 1 B, Text-fig. 3 and 4). The egg remains a broken shape for about an hour and a half, or until the test-cells are expelled. As the enveloping membranes swell, and as the test-



Text-fig. 1. Illustration of maturation in the living egg of *Cynthia roretzi* DRASCHE. $\times 130$. (A) side view of oöcyte immediately after ovulation, (B) same, 1 hour after, (C) same, 2 hours after, (D) same, 3.50 hours (E) same, 4.58 hours after. *c* chorion, *dr* dark region, *f* follicular membrane, *g* germinal vesicle, *lr* light region, *ps* perivitelline space, *t* test-cells.

cells are expelled from the oöcyte and become free in the perivitelline space, the oöcyte becomes a nude egg and its shape reverts to the spherical form (Text-fig. 1 C). After about three and half hours the egg becomes flattened at the animal pole and becomes somewhat elongated,



Text-fig. 2. Successive illustration of Text-fig. 1. $\times 130$. (F) lateral view of secondary oöcyte about 4.90 hours after ovulation, (G) same, 5.0 hours after, (H) same, 5.1 hours after, (I) same, 5.2 hours after, (J) same, 5.3 hours after, (K) posterior side view of ovum, 5.5 hours after, (L) 2 cell-stage of egg, 5.6 hours after. *1pb* 1st polar body, *2pb* 2nd polar body.

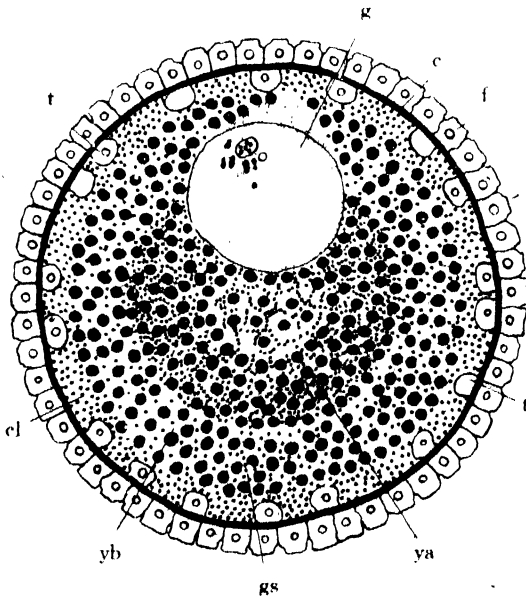
and the extrusion of the first polar body follows (Text-fig. 1 E). Then it returns to a spherical shape. After about four hours and fifty minutes, again the animal pole becomes flattened and the second polar body is formed (Text-fig. 2 F). As Text-fig. 1 H—1 L shows, some changes of the egg-shape followed in the latter period until the first cleavage.

THE FIXED EGG

a. Before the Rupture of the Germinal Vesicle

In the fixed egg which corresponds to the Text-fig. 1 A, there are a distinct germinal vesicle near the pole and a large amount of egg-plasm, rich in yolk granules. In the sections of the egg fixed by CHAMPY's mixture and stained by MALLORY's connective tissue staining, the nuclear membrane and the granular contents of the nucleus are stained with acid fuchsin. There are a large nucleolus and two or three small ones, and

they take the stain of Orange G. There are also several particles stained with acid fuchsin, which may be called the gemini. In the sections stained with HEIDENHEIN's iron-haematoxylin, the so-called gemini and nucleolus take the dense stain of haematoxylin, but the nuclear membrane and granular contents are stained to a less degree by this colour. The membrane and granular contents are also stained with ALTMANN's acid fuchsin by CHAMPY-KÜLL's triple staining method.



Text-fig. 3. Drawing of sectioned primary oocyte, showing its structure. $\times 220$. *c* chorion, *cl* substance in cortical layer, *f* follicular epithelium, *g* germinal vesicle, *gs* granular substance which occupy spaces between yolk granules, *t* test-cells, *ya* central yolk mass, *yb* peripheral yolk mass.

The cytoplasm which corresponds to the dark region in the living egg,

consists of two varieties of yolk-masses. One of them indicated by (ya) in Text-fig. 3 is a concentric sphere at the centre of the egg. And the other indicated with (yb) surrounds the former. Yolk granules in the former take the stain of anilin blue, but are not stained with the haematoxylin, but those of the latter take the stain of the Organe G, or of the haematoxylin. The space surrounding these yolk-granules is filled with fine granules which take the same stain as the cortical substance (Text-fig. 3, gs).

The cortical part, which corresponds to the light region in the living egg, contains no yolk granules but finely granular substances. These substances are stained violet by MALLORY's staining method, but are not stained with the haematoxylin or with the ALTMANN's acid fuchsin (Text-fig. 3, cl).

The chorion stained with anilin blue adheres closely to the surface of the oöcyte as stated in the observation of the living egg. The primary egg-membrane cannot be distinguished from the chorion even in the fixed and stained specimens (Text-fig. 3, c).

The content of the nucleus, with the exception of the gemini, was called the "residual substance" by F. R. LILLIE (1906) in *Chaetopterus pergamentaceus* CUVIER. In the same manner, the present writer also applies the term "residual substance" to the egg of *Cynthia roretzi*. The residual substances and the cortical substance play an important rôle in the development. In this respect, the sections fixed by CHAMPY's mixture and stained with MALLORY's connective tissue-staining received the best method to distinguishing these two substances. By this method, the residual substance always stains differently from the cortical substance, viz. the former is red and the latter violet. Specimens fixed by ZENKER's mixture and the other fluids showed neither the distinction between the natures of the nuclear substance and those of the cortical plasm, nor the distinction between the natures of the two varieties of yolk-granules.

Thus, the structure of the egg is apparently radially symmetrical before the maturation.

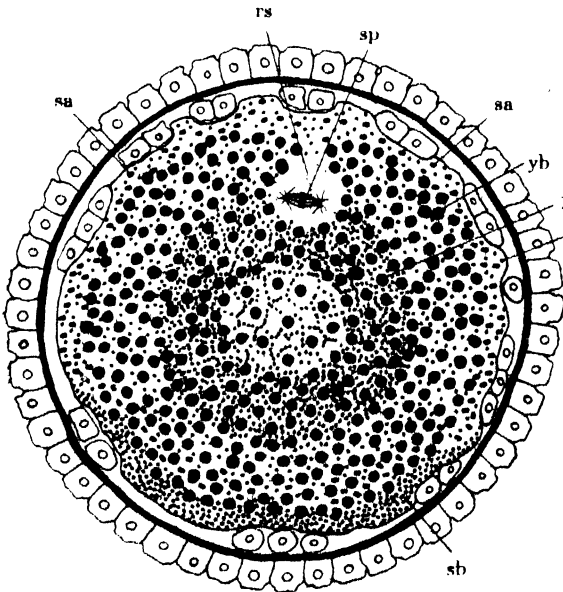
b. *Maturation and Fertilization*

1) RUPTURE OF THE GERMINAL VESICLE

If the egg of *Cynthia roretzi* be taken and allowed to stand in seawater, the germinal vesicle breaks down, whether the egg be fertilized or

not. The process of the rupture of the germinal vesicle can be seen in the egg which has been standing about an hour in the sea-water. At the beginning of the rupture, as the germinal vesicle decreases in size, a granular substance, the nature of which resembles that of the above-mentioned granular substance of the germinal vesicle in the resting period, begins to gather inside the nuclear membrane, and the membrane begins to increase in thickness. Next the granular, thickened structure of the membrane becomes coarse, and at last the membranous structure dissolves. These observations of the nucleolus and chromosomes are incomplete, the present paper being only an outline with regard to them. Meanwhile, the gemini gather at a point and the aster appears there. This is the prophase of the first maturation division. The germinal spots and a part of the residual substance surround the gemini and the aster. A spindle now appears parallel to the tangential plane of the oöcyte, and the chromosomes arrange themselves at the middle of the spindle. This

is the metaphase of the first maturation division. These processes take place at the position in which the germinal vesicle was placed in the yolk mass area (Text-fig. 4, *sp*).



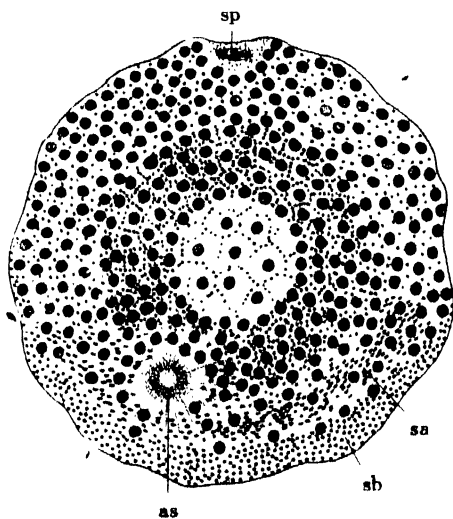
Text-fig. 4. Drawing of sectioned primary oöcyte at metaphase of first maturation division, showing changes of nuclear substance, and of cortical layer-substance. $\times 220$. *rs* residual substance of germinal vesicle, *sa* nuclear substance (*sa*), *sb* substance in cortical layer (*sb*), *sp* metaphase of first maturation spindle, *ya* central yolk mass, *yb* peripheral yolk mass.

The rupture of the germinal vesicle initiates a series of movements of the egg substances during the prophase of the first maturation division. Following the rupture of the germinal vesicle, the residual substance, which is stained with the acid fuchsin in MALLORY's connective tissue staining, begins to move towards the pole of the egg, passing through

the bottle-neck passage through the yolk layer, until it reaches the cortical layer. At the metaphase of the first maturation division, the red residual substance, which is stained with the acid fuchsin mentioned above, is found in the cortical layer of the upper two-thirds of the egg (Text-fig. 4, sa). At the same time, most of the violet substance, which completely covered the oöcyte before the breaking of the germinal vesicle, moves to the cortical part of the antipole (Text-fig. 4, sb). The red residual substance is composed of rod-shaped, fine granules which are a little larger than those of the cortical layer stained in violet (Text-fig. 4, as and sb). In the sections stained with CHAMPY-KULL's triple staining, the rod-shaped granules, which take the stain of ALTMANN's acid fuchsin, are also found in the area of the residual substance.

2) ENTRANCE OF THE SPERMATOZOÖN AND FUSION OF MALE AND FEMALE PRONUCLEI

The first maturation spindle in metaphase which has been laid in the area of the yolk mass, moves to the surface of the oöcyte with the flow of the residual substance, passing through the bottle-neck passage. This passage disappears after the spindle has passed, and is then replaced by the yolk granules. At this period the above mentioned violet substance (sb) moves to one-third of the peripheral part of the antipole. At the same time, most of the above-mentioned red substance (sa) moves to mix with the yolk granules at the upper zone of the violet substance, but a small portion of the red substance is left around the spindle. And then, the upper two-thirds of the cortical layer of the egg are replaced by the yolk granules. The consistency of the yolk



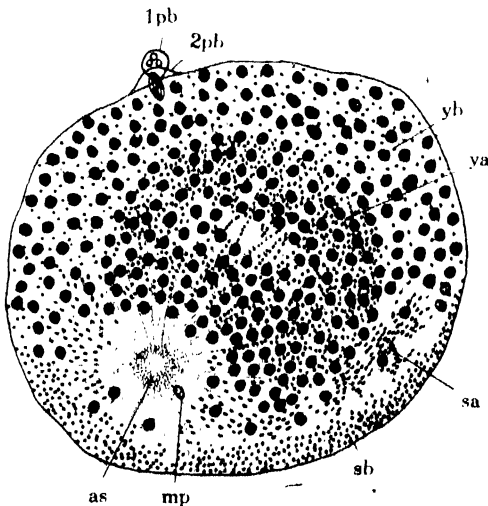
Text-fig. 5. Drawing of sectioned primary oöcyte at period of entry of spermatozoön, showing changes of nuclear and cortical layer substances. $\times 220$. *as* astrosphere of male pronucleus, *sa* substance (*sa*), *sb* substance (*sb*), *sp* spindle of metaphase of first maturation division.

granules at the zone where the red substance (sa) exists is much rougher in comparison with the other yolky part. At this stage, therefore, there exist two kinds of cytoplasmic layers at about one-third of the oöcyte at the antipole.

The entry of the spermatozoön occurs invariably at the metaphase of the first maturation division, without regard to the position of the maturation spindle and to the structures of the cytoplasm. The spermatozoön enters the oöcyte at the region near the vegetative pole of the egg, and soon after the entered spermatozoön develops a well-marked aster (Text-fig. 5, as). The sperm nucleus with its aster at first moves towards the centre of the egg, continuing until it has traversed the violet substance (sb) and has reached the red substance (sa). This is the penetration path. The male pronucleus with its astrosphere moves towards the pole along the inner surface of the oöcyte passing through the peripheral yolk zone, increasing in size (Text-fig. 6, mp and as). The maturation spindle which has been laid parallel to the tangential plane of the oöcyte rotates in the radial position. After the entry of the spermatozoön, the

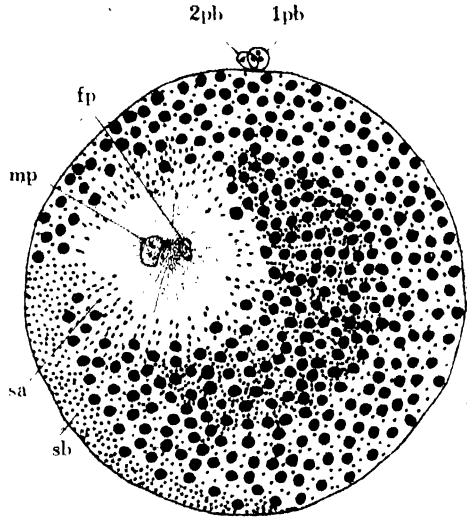
first polar body is cut off, and then the second polar body is formed (Text-fig. 6, 1pb and 2pb). After the formation of the second polar body, the female pronucleus descends from the pole to unite with the male pronucleus through the yolk zones, recovering its volume. When the male pronucleus and its astrosphere reach a point a little above the equator, they change their direction of movement and proceed towards the centre of the oöcyte (Text-fig. 7, mp and fp). This is the copulation path, the male pronucleus uniting with the female pronucleus at a point near the centre (Text-fig. 7, mp and fp).

The movement of the male



Text-fig. 6. Drawing of sectioned secondary oöcyte, showing male pronucleus, its astrosphere, and change in cytoplasm. $\times 220$. as astrosphere of male pronucleus, mp male pronucleus, 1pb 1st polar body, 2pb 2nd polar body, sa substance (sa), sb substance (sb), ya central yolk mass, yb peripheral yolk mass.

pronucleus initiates the second movements of the cytoplasm. Soon after the entry of the spermatozoön, the red substance (sa) is found also around the astrosphere (Text-fig. 6, sa). Following the movement of the male pronucleus, the red substance (sa) and the violet substance (sb) move in an upper direction along the curvature of the oöcyte as if they were drawn by the male pronucleus and its astrosphere. As the male pronucleus turns inwards, it draws some of the two substances with it, but leaving most of the violet substance (sb) behind (Text-fig. 7, sa and sb). Not only these two substances but also the yolk masses change their shapes. The two yolk masses were concentric spheres before the entry of the spermatozoön (Text-fig. 6 and 7, ya and yb), but they change their shape into crescentric masses, which cover the nuclei and astrospheres at the opposite side of the red and violet substances (sa and sb). The movements of these substances are now completed. In this structure of the cytoplasm, the oöcyte is now ready to undergo the first cleavage.

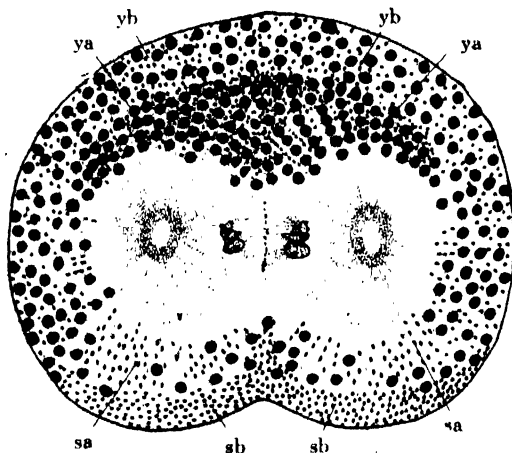


Text-fig. 7. Drawing of sectioned ovum at period of fusion of male pronucleus with female, showing changes in cytoplasm. $\times 220$. fp female pronucleus, mp male pronucleus, 1pb 1st polar body, 2pb 2nd polar body, sa substance (sa), sb substance (sb).

c. First Cleavage

Just before the fusion of the two pronuclei, the astrosphere with its central body and aster of the male pronucleus divides into two equal halves at right angle to the copulation path, and they recover their spherical forms when the division of the fused nuclei occurs. The first meridional cleavage furrow occurs, at first, at the side of the egg where the red and violet substances (sa and sb) exist, so as to divide these substances into two halves, and the furrow extends to all over the egg and divides it into two precisely equal cells (Text-fig. 8).

The structure of the egg was radially symmetrical before the entry of the spermatozoön, but it becomes bilaterally symmetrical after the



Text-fig. 8. Drawing of equatorially sectioned ovum, in telophase of first cleavage dividing ovum into two equal cells. $\times 220$. sa substance (sa), sb substance (sb), ya central yolk mass, yb peripheral yolk mass.

movement of the male pronucleus initiates the secondary movements of the cytoplasm.

GENERAL CONSIDERATION

It is a well-known fact that the eggs of many forms change their shape markedly in the period between the time of insemination and first cleavage. Many observers have studied this problem in *Chaetopterus* and other annelids, mollusks, coelenterates, etc. In the *Nereis* egg, LEIGH HOADLY ('34) stated that after insemination the egg shows a series of deformations which are continuous but which decrease in extent up to the time of the second cleavage. ARTHUR COHEN and N. J. BERRILL ('36) observed the changes in the egg-shape of *Ascidella aspersa* from fertilization to the first cleavage, and stated as follows "The changes start with a flattening of the egg about two minutes before the first polar body is extruded. About three minutes after the latter event the egg returns to its spherical shape. Shortly afterwards the egg undergoes a series of deformations which last until the first cleavage, being interspersed with periods during which the egg returns to a spherical condition. The changes in shapes indicates that the extremely delicate egg membrane nevertheless possesses a high degree of elasticity." The present writer

observed the deformation in the egg-shape of *Cynthia roretzi* during the period of maturation and the first cleavage. The change in the egg-shape start with the beginning of the maturation and it continues until the first cleavage, while the violent changes are taking place in the distribution of the substances of the nucleus and cytoplasm, as mentioned already in the chapter recording the observations.

E. G. CONKLIN ('05) stated that the fate of the test-cells in *Cynthia partita* is that they are expelled from the cytoplasm of the egg when the ripe egg is discharged from the ovary, and that they lie in the perivitelline space, which is formed between the chorion and the egg-cell by the shrinkage of the latter. In the egg of *Cynthia roretzi*, the first maturation division begins about an hour after the egg is placed in the sea-water. The flow of the egg substances and the change of the egg-shape start with the maturation of the nucleus. During this period, the test-cells are expelled from the cytoplasm of the egg. Finally they are liberated in the space between the egg-cell and the chorion.

The cytoplasmic movement in the maturation period is an important event in the maturation of egg. T. H. MORGAN ('27) stated that "Extensive movements of the materials of the egg are known to take place in some eggs just prior to the division. In other eggs, where such movements have not been described, it is not improbable that similar, if less extensive changes, take place." The movements of oöplasms were observed by H. DRIESCH ('97) in *Myzostoma*, TH. BOVERI ('01) in *Strongylocentrotus lividus*, E. B. WILSON ('04) in *Dentalium*, F. R. LILLIE ('06) in *Chaetopterus*, E. G. CONKLIN ('10) in *Physa heterostrophia*, *Lymunaea columella*, and *Planorbis trivolvis*, WEIGMANN ('27) and I. MOTOMURA ('35) and etc. in Amphibia, and I. MOTOMURA in *Strongylocentrotus purcherrimus* and etc. In Ascidiae, E. G. CONKLIN ('05) studied carefully this problem in the egg of *Styela (Cynthia) partita*. After being laid, the egg of this species begins to undergo maturation changes. The clear nuclear sap flows upwards and forms a cap of clear cytoplasm at the animal pole of the egg. The spermatozoön enters the egg at the metaphase of first maturation division, and immediately after fertilization the downward-streaming of the yellow cytoplasm and the outflow of the contents of the germinal vesicle occurs. These streaming substance form a mass of clear cytoplasm and a yellow crescent at the posterior side of the egg. In *Cynthia roretzi*, the behaviour of the nuclear substance and the cytoplasm in the course of maturation nearly resemble those of *Styela partita*, except that the clear peripheral layer of the living egg of the

former species is destitute of the pigment substance. And it is, therefore, hard to distinguish the substance of the germinal vesicle from that of clear peripheral layer in the living egg. The present writer tried several staining methods in order to distinguish these substances in the sections, and he found that in the materials fixed with CHAMPY's mixture and stained with MALLORY's connective tissue staining method, the substance of the germinal vesicle stained red with acid fuchsin, and the substance of the peripheral layer violet with acid fuchsin and aniline blue combined. These characteristics of the staining reaction make it possible to observe the movement of the substances. In the egg of *Cynthia partita*, the movements of the cytoplasm begin immediately after the entrance of the spermatozoön, but in the case of the egg of *Cynthia roretzi*, the first movements of the cytoplasm seem to begin prior to the fertilization. This is also the case in the egg of *Chaetopterus* (F. R. LILLIE) in which the polarization as a result of the movements of the cytoplasm is independent of fertilization.

The nuclear sap is generally regarded as inaugurating the cytoplasmic movements. There are many records of the existence of a large quantity of residual matter derived from the germinal vesicle after the formation of the first maturation spindle. F. R. LILLIE ('06) stated "CONKLIN's observations ('05) are most complete ones on the subject of the residual substance of the germinal vesicle..... It seems probable, therefore, that the residual substance of the germinal vesicle represents a specific formative stuff of essentially the same character in different phyla". The present writer observed the fate of the residual substance stained with acid fuchsin in the maturation period of the egg of *Cynthia roretzi*. He considers that the nuclear sap of the germinal vesicle is not only important in respect of its being formative stuff, but also as playing some roles in the cytoplasmic maturation of the egg.

In certain eggs polar differentiation is shown, not only by the eccentricity of nucleus, but also by the stratification of egg-substances at right angles to the polarity. In some eggs, the stratification of egg substances arises before maturation, and in other eggs it arises after maturation. In the egg of *Cynthia roretzi*, the polar differentiation is shown by the eccentricity of nucleus, and also by the stratification of egg-substances, which arise at the stage of maturation. Up to the time of fertilization all the egg-substances are radially symmetrical. But after the male and female pronuclei unite with each other and the egg substances flow to one side of the egg, the egg structure shows a bilateral symmetry,

In the egg of *Cynthia partita*, the first cleavage furrow divides the egg into equal right and left halves, and this is also the case in the egg of *Cynthia roretzi*.

SUMMARY

1. The observations on the early development of the egg of *Cynthia roretzi* DRASCHE were carried out by means of artificial insemination in HERBST's ('04) artificial sea-water.

2. The follicular envelope with the chorion swells during the maturation of the oöcyte, and at the same time the test-cells are expelled and become free in the perivitelline space. The development of the egg progresses inside this membrane.

3. The deformation of the egg-shape is observed in the period from the beginning of maturation to the first cleavage.

4. The structure of the primary oöcyte is radially symmetrical before maturation. A large nucleus exists eccentrically to the pole. There are also cortical substance and two spherical zones of central yolk-masses. And there are fine granules which occupy the spaces of these yolk granules. In the specimens killed in CHAMPY's fluid and stained with MALLORY's connective tissue staining, the cortical substance is stained in violet and the "residual substance" of the germinal vesicle in red.

5. Whilst the maturation divisions have been proceeding, the particular movements of the cytoplasm take place in the egg. The primary movement of the oöplasm occurs during the first maturation division. The movement of the male pronucleus, which enters the oöcyte at the anti-polar region at the metaphase of the first maturation division, initiates the secondary movements of the cytoplasm. By these movements of the oöplasm, the radially symmetrical structure of the egg changes into the bilaterally symmetrical structure.

6. The "residual substance" of the germinal vesicle flows out and takes part in the cytoplasmic movements in the egg.

7. The first cleavage furrow is formed so as to divide the structure of the oöcyte into two precisely equal halves.

PUBLICATIONS REFERRED TO

- BOVERI, TH., 1901. Die Polarität von Ovocyte, Ei und Larve des *Strongylocentrotus lividus*. Zool. Jb. Abt. F. Anat., Bd. 14.
- COHEN, A. and N. J. BERRILL, 1936. The Early Development of Ascidian Eggs. Biol. Bull., Vol. LXX.
- CONKLIN, E. G., 1905. Organization and Cell-lineage of the Ascidian Egg. vide COWDRY and other text-books.
- CONKLIN, E. G., 1910. The Effect of Centrifugal Force upon the Organization and Development of the Eggs of Fresh Water Pulmonates. J. Exper. Zool., Vol. 9.
- COWDRY, E. V., 1924. General Cytology. Chicago.
- DRIESCH, H., 1897. Betrachtung über die Organization des Eies und ihre Genese. Roux' Archiv, Bd. 4.
- HIRAI, E., 1939. A Report of Observations made of the Egg-follicle Formation in the Case of *Cynthia roretzi* DRASCHE. Sci. Rep. Tōhoku Imp. Univ. Biol., Vol. XIV.
- HOADLEY, L., 1934. Pulsations in the *Nereis* Egg. Biol. Bull., Vol. LXVII.
- LILLIE, F. R., 1906. Observations and Experiments Concerning the Elementary Phenomena of Embryonic Development in *Chaetopterus*. J. Exper. Zool., Vol. 3.
- MOTOMURA, I., 1935. Notes on the Cytoplasmic Structure of the Egg of a Sea-urchin, *Strongylocentrotus*. Zool. Mag., Vol. 48.
- MOTOMURA, I., 1935. Determination of the Embryonic Axis in the Eggs of Amphibia and Echinoderms. Sci. Rep. Tōhoku Imp. Univ., Biol., Vol. X.

昭和十六年四月二十五日印刷
昭和十六年四月三十日發行

定價金貳圓

編輯兼發行者 東北帝國大學

東京市本郷區駒込林町百七十二番地

印刷人 柴 山 則 常

東京市本郷區駒込林町百七十二番地

印刷所 合資 杏 林 舍

東京市日本橋區通三丁目

賣捌所 丸 善 株 式 會 社

仙臺市國分町

賣捌所 丸 善 株 式 會 社 仙臺支店

CONTENTS

KATSUHIRO OKADA

The Gametogenesis, the Breeding Habits, and the Early Development of *Arenicola cristata* Stimpson, a Tubicolous Polychaete. . . 99

SHINJIRO KOBAYASHI

Earthworms of Korea. II. 147

MITUO SATO

A Comparative Observation of the Hind-Brain of Fish Possessing Barbels, with Special Reference to their Feeding Habits. 157

NOBUO SASAKI

The Fresh-water Sponges of Tisima-Rettô (the Kurile Islands). . . 165

YOSINORI SUGIHARA

The Embryogeny of *Cunninghamia lanceolata* HOOKER. 187

MAKOTO TORIUMI

Studies on Freshwater Bryozoa of Japan. I. 193

ETURO HIRAI

The Early Development of *Cynthia roretzi* DRASCHE. 217

All communications intended for "THE SCIENCE REPORTS, FOURTH SERIES" should be addressed to Prof. M. TAHARA, the Biological Institute, Tôhoku Imperial University, Sendai, Japan.

SOME FRESHWATER BRYOZOA FOUND IN MANCHOUKUO

By

SANJI HÔZAWA AND MAKOTO TORIUMI

Biological Institute, Tôhoku Imperial University, Sendai, Japan

(With Plate XIV and 7 text-figures)

(Received November 20, 1940)

INTRODUCTION

The fauna of the freshwater Bryozoa of Manchoukuo has remained entirely unknown up to the present time. Two papers, one dealing with *Lophopodella carteri* by OKA and the other on the forms existing in Northern China by LEE are the only references which the writers were able to find. They are as follow:—

1. OKA, 1911, Report on the Freshwater Bryozoa (Tansui Kokemusi Hôti). Zool. Mag. (Dôbutugaku Zassi) Vol. 23, p. 588.
2. LEE, 1936, Notes on some Freshwater Polyzoa of Peiping. Sinen-sia, Vol. 7, pp. 399-407.

In 1938 Prof. KAWAMURA and Dr. MIYAJI of the Kyôto Imperial University visited to Manchoukuo at great risk, and obtained many freshwater animals. The specimens of the freshwater Bryozoa were kindly forwarded to the writers for identification.

The present report dealt with this material and forms determined are shown in the following list.

1. *Plumatella repens* var. *fungosa* (PALLAS)
2. *P. repens* var. *emarginata* (ALLMAN)
3. *P. repens* var. *annulata* HÔZAWA & TORIUMI
4. *P. auricomis* ANNANDALE
5. *Plumatella* sp.
6. *Plumatella* sp.
7. *Hyalinella toanensis* HÔZAWA & TORIUMI

DESCRIPTION OF FORMS

- 1) *Plumatella repens* var. *fungosa* (PALLAS)

Tubularia fungosa, PALLAS, 1768.

Alagonella fungosa, ALLMAN, 1856, p. 87, Pl. III, figs. 1-7.

Plumatella polymorpha var. *fungosa*, KRAEPELIN, 1887, p. 124, p. 125, Pl. IV, fig. 112, Pl. VII, figs. 140-142; DAVENPORT, 1904, p. 218.

Plumatella repens var. *fungosa*, VANGEL, 1894, p. 153; ANNANDALE, 1910, p. 44; HÔZAWA & TORIUMI, 1910, p. 426, Fig. 1.

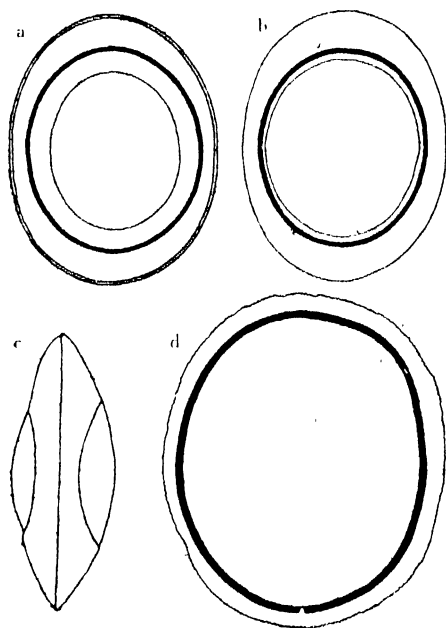
Plumatella fungosa, BRAEM, 1890, pp. 3-8, Pl. I, figs. 2-6, Pl. II, figs. 16-20; HARTMEYER, 1909, p. 54, Fig. 120; LEE, 1936, p. 405, Fig. 4.

Zoarium. It is fungoid and dark grey in colour. As the specimens are dried, the zoaria become solid. The larger of the two specimens received is about 15 cm in length and the smaller is about 5 cm long.

Zooecia. The zooecia are set exactly parallel to one another and are agglutinated together, thus assuming a polygonal shape in cross section.

Polypides. It cannot be examined as the zoaria are dried.

Statoblasts. Both fixed and free statoblasts (text-fig. 1) are present in great numbers.



Text-fig. 1. Statoblasts of *Plumatella repens* var. *fungosa*. a-c, free statoblasts; d, fixed statoblasts. $\times 100$.

The former are broadly oval in form, and are surrounded by a stout and serrated chitinous lamella which shows obscure irregular reticulation from vestigial air-cells.

The capsule is minutely mammillated. As a rule, some of the fixed statoblasts are seen adhering to the substratum arranged in a row. The length of the fixed statoblasts is about 0.8 mm and the breadth about 0.39 mm.

The free statoblasts are also broadly oval being 0.35-0.37 mm long and 0.21-0.23 mm wide.

The capsule of free statoblast shows a mammillation.

Remarks. The material was already dried when it was collected.

Locality. Mukden City

2) *Plumatella repens* var. *emarginata* (ALLMAN)

Plumatella emarginata, ALLMAN, 1844; 1856, p. 104, Pl. VII, figs. 5-10; BRAEM, 1890,

pp. 9-10, Pl. I, figs. 9, 12, 14; HARTMEYER, 1909, p. 53, Fig. 117; ANNANDALE, 1910, p. 47; VORSTMAN, 1928, p. 4, Figs. 1, 2, Pl. I, figs. 1-4; HASTINGS, 1929, p. 137, p. 307.

Plumatella princeps var. *emarginata*, KRAEPELIN, 1887, p. 120, Pl. IV, fig. 108, Pl. V, fig. 123; DAVENPORT, 1904, p. 217, Pl. VI, fig. 5.

Plumatella emarginata forma *typica*, LEE, 1936, pp. 401-403, Fig. II.

Plumatella repens var. *emarginata*, VANGFL, 1894, p. 154; ROGICK, 1935, pp. 255-256, Pl. XLI, fig. 6; 1937, pp. 100-101; 1940, pp. 198-200, Pl. I, Figs. 1-3, Pl. III, fig. 11, 12; HÔZAWA & TORIUMI, 1940, p. 427, Fig. 2, Pl. fig. 7.

Zoarium. The zoarium branches in an antler-like manner and it appears that these branches were almost free from the substratum. The ectocyst is membranous being slightly encrusted and is pale yellowish-brown in colour and transparent.

Zooecia. The zooecia are long, nearly cylindrical and are sometimes feebly keeled.

The notched margin is not observable as the ectocyst is slightly encrusted and is transparent. Some incomplete septa, each in the form of a crescent are attached to the upper wall of the zoarium. They are either deep brown or black in colour.

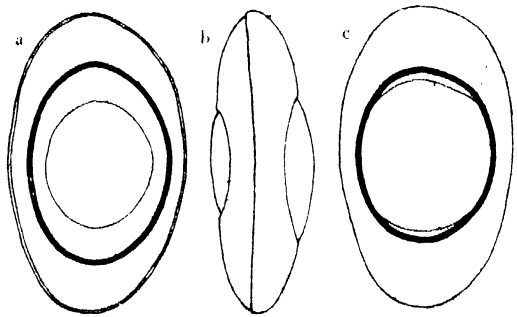
Rarely, the septum occurs at the end of each zooecium.

Polypides. Most of the polypides have disappeared. The number of the tentacles is from 36 to 42.

Statoblasts. Only free statoblasts (text-fig 2) are present. Its length is 0.37-0.42 mm, and the breadth is 0.21-0.24 mm. The capsule has a mammillation. The annulus covers the greater part of one surface, namely the dorsal side of the capsule and a small part of the other surface.

Usually, the annulus is much narrower at the sides than at the extremities. The capsule is 0.25-0.28 mm long and 0.18-0.2 mm wide.

Remarks. The specimen above mentioned was difficult to identify because the statoblast was somewhat different from that of typical *emarginata* showing an encroachment of annulus slightly greater on one side of the capsule than the other. After much consideration, this form



Text-fig. 2. Statoblasts of *Plumatella repens* var. *emarginata*. $\times 100$.

was delegated to *Plumatella repens* var. *emarginata*.

Locality. Dairen City

3) *Plumatella repens* var. *annulata* HÔZAWA & TORIUMI

Plumatella repens var. *annulata*, HÔZAWA & TORIUMI, 1940, pp. 249-250, Fig. 3, Pl. figs. 2, 3.

Zoarium. The whole zoarium is recumbent, the branches overlapping one another, consequently the zooecia are greatly crowded. The ectocyst is thick, stiff and brown in colour, but in the newly formed zooecia it is thin, soft, sometimes encrusted and is of a sandy colour. All the zooecia, except the newly formed ones, have an annulation which is formed by the ectocyst thickening in a ring-like manner.

The annulation is very conspicuous in the distal half of the zooecium.

Zooecia. The zooecium is short and its base is recumbent. The distal part of each zooecium is bent upwards and is free, while the newly formed zooecium is entirely recumbent. Each zooecium terminates abruptly and is truncated. In the newly formed zooecium the tip is rounded off, as is the case in most of the species of the genus.

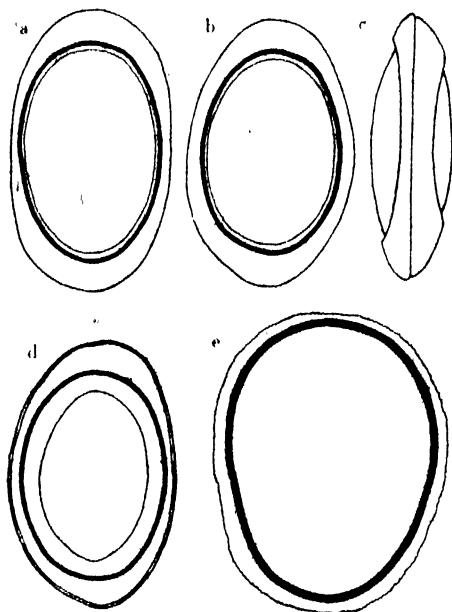
On the dorsal surface of each zooecium there is a conspicuous keel extending to the distal end.

Polypides. The number of the tentacles varies from 21 to 28.

Statoblasts. The free statoblasts (text-fig. 3, a-d) are elongated. The annulus covers a small part of both surfaces of the capsule and is much broader at the ends than at the sides.

The capsule has a mammillation on each surface. The length of the free statoblasts is 0.35-0.37 mm and the breadth is 0.21-0.23 mm.

The capsule is 0.28-0.29 mm long and 0.18-0.2 mm wide.



Text-fig. 3. Statoblasts of *Plumatella repens* var. *annulata*. a, b, ventral; c, side; d, dorsal view of the free statoblasts; e, fixed statoblast. $\times 100$.

The fixed statoblast (text-fig. 3, e) is oval in shape and is surrounded by chitinous lamella. The length of the fixed statoblasts is 0.37–0.4 mm and the breadth is 0.25–0.28 mm. The caspule of the fixed statoblast has a mammillation.

Remarks. This variety was found growing on the shells of a small gasteropod and on the stem of a water-plant.

In the former case, the zoaria were found covering the entire shell except the operculum.

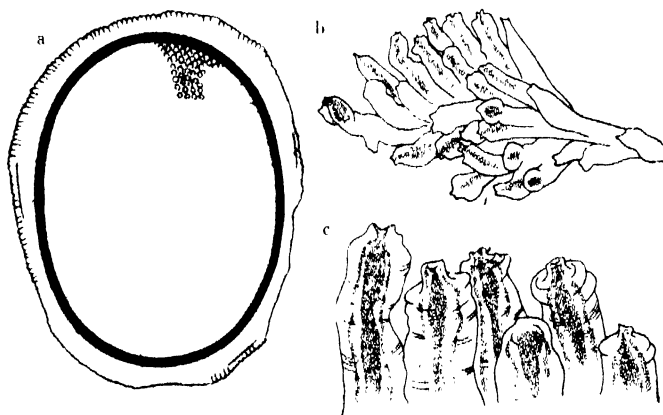
This variety resembles *Plumatella tanganyikae* ROUSSELET but differs from it in the form of the free statoblast. In this variety, the annulus is much narrower at the sides than at the ends.

Locality. Mu-tan-k

4) *Plumatella auricomis* ANNANDALE

Plumatella auricomis, ANNANDALE, 1913, p. 223, Fig. 2; HÔZAWA & TORIUMI, 1940, p. 429, Fig. 4, Pl. fig. 8.

Zoarium. The zoarium is small and entirely recumbent with short horizontal branches closely set (text-fig. 4, b). The ectocyst is greatly



Text-fig. 4. *Plumatella auricomis*. a, fixed statoblast (dorsal view) $\times 100$; b, a portion of the zoarium; c, distal parts of the zooecia.

thickened, and is either colourless or more or less a pale yellowish-brown hue. It is hyaline and stiff differing from the ectocyst of *Hyalinella punctata* which is soft and contractile. The ectocyst forming the distal part of the zoecium is wrinkled (text-fig. 4, c).

Zooecia. The zooecia are short, stout, L-shaped and cylindrical without any trace of dorsal keel or furrow. Rarely, a septum is present at the base of the branch.

Polypides. The tentacles are from 37 to 42 in number.

Statoblasts. No free statoblasts are observed. The fixed statoblasts (text-fig. 4, a) are 0.44–0.47 mm long and 0.35–0.37 mm wide and possess the chitinous lamella. The capsule has a mammillation on its dorsal surface.

Remarks. It was found growing on a molluscan shell.

Locality. Lake Bôr (Buir Nor)

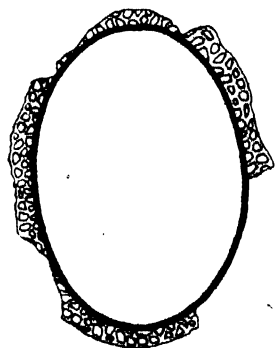
5) *Plumatella* sp.

Zoarium. It is small, antler-like in form and is entirely recumbent. The ectocyst is encrusted and is of a sandy colour.

Zooecia. The zooecia are cylindrical in form and the distal part of each zoecium is never bent upwards. The ectocyst, forming the distal part of the zoecium, is pigmented with a dark grey colour. A furrowed keel is found on the dorsal surface of zoecium. The septa are not observed.

Polypides. As the polypides have almost disappeared the tentacles are not easily examined. In one case they were 21 in number while in another they were 30.

Statoblasts. Only the fixed statoblasts (text-fig. 5) are sparingly produced. Each statoblast is about 0.4 mm long and about 0.28 mm broad.



Text-fig. 5. Fixed statoblast of *Plumatella* sp. $\times 100$.

The chitinous lamella shows well-defined, irregular reticulation from the vestigial air-cells. The edge of the lamella has no serration. The capsule is minutely mammillated.

Remarks. It was not possible to identify the species owing to the fact that the material was insufficient in quantity.

Locality. Mishanfu

6) *Plumatella* sp.

Of this form nothing was found except some fixed statoblasts (text-

fig. 6) adhering to a stone, and they do not show any of the characteristics necessary for the identification of a species.

Locality. Lake Kulun (Dalai Nor)

7) *Hyalinella toanensis* HÔZAWA & TORIUMI

Hyalinella toanensis, HÔZAWA & TORIUMI, 1940, p. 431, Fig. 6, Pl. fig. 4.

Zoarium. It forms a nodular mass growing around the stem of some water-plants. The surface is generally not smooth owing to the conical or rounded tips of the zooecia. The jelly is copious, and sometimes it attains to a thickness of 1 cm. It is colourless and hyaline. When preserved in spirit it becomes somewhat hard and elastic. In the mode of branching, the zoarium shows some features similar to those seen in the case of *Hyalinella indica* ANNANDALE.

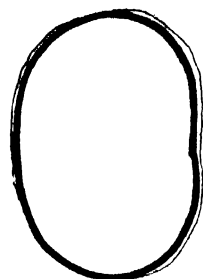
Zooecia. The zooecium does not take the figure-L form. It is either straight or slightly bent upwards, the tip being projected from the coenoeccial jelly.

Polypides. The number of the tentacles is between 50 to 73. The calyx is distinctly festooned.

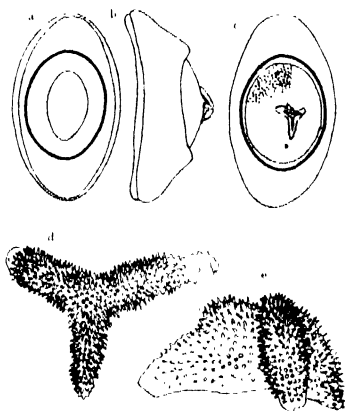
Statoblasts. The free statoblasts (text-fig. 7) are somewhat rhombic (sometimes elongated) and are rounded at both extremities. The annulus encroaches on the capsule a little more on one surface than the other. The annulus is not curved as in the case of *H. indica*. The capsule has a large blunt process in the centre of the ventral surface, thus the dorsal and ventral surfaces are asymmetrical when observed in section. The blunt process is provided with a spinous, hyaline appendage (text-fig. 7, d, e) which may be easily detached from the process.

The capsule has a number of minute tubercles scattered on both surfaces.

The length of the free statoblasts



Text-fig. 6. Fixed statoblast of *Plumatella* sp. $\times 100$



Text-fig. 7. Statoblasts of *H. toanensis*. a-c free statoblasts $\times 50$. d, e spinous appendages of free statoblast (d upper view, e side view) $\times 270$.

ranges from 0.5 to 0.6 mm and the breadth is from 0.3 to 0.37 mm.

The capsule is from 0.31 to 0.39 mm long and is from 0.24 to 0.3 mm broad. No fixed statoblasts were observed.

Remarks. The species bears a close resemblance to *Hyalinella indica* ANNANDALE from India, differing only in the characteristics of the statoblast and in the shape of the zooecium.

Locality. Mishanfu.

LITERATURE CITED

- ALLMAN, G. J., 1856. "A Monograph of the Fresh-Water Polyzoa." Ray Soc. London.
- ANNANDALE, N., 1910. Materials for a revision of the Phylactolaematous Polyzoa of India. Rec. Ind. Mus., Vol. 5, pp. 37-57.
- , 1913. A report on the Biology of the Lake of Tiberias. Sec. Ser. The Polyzoa of the Lake of Tiberias. Jour. Proc. Asia. Soc. Bengal. Vol. 9, pp. 223-228.
- , 1915. The Genus *Australella* and some allied species of Phylactolaematous Polyzoa. Rec. Ind. Mus., Vol. 11, pp. 163-169.
- BRAEM, F., 1890. "Untersuch. über die Bryoz. des süßen Wassers." Bibliotheca Zoologica, Heft VI.
- DAVENPORT, C. B., 1904. Report on the Fresh-Water Bryozoa of United States. Proc. U. S. Nat. Mus. Vol. 27, pp. 211-221.
- HARMER, S. F., 1913. The Polyzoa of Waterworks. Proc. Zool. Soc. London. 1913. II, pp. 426-457.
- HARTMEYER, R., 1909. "Bryozoen, Moostierchen," in A. Brauer, 'Die Süßwasserfauna Deutschlands' Heft 19, pp. 49-58.
- HASTINGS, A. B., 1929. Notes on some little-known Phylactolaematous Polyzoa and description of a new species from Tahiti. Ann. Mag. Nat. Hist. ser. X, No. 3, pp. 300-310.
- HÔZAWA, S. & TORIUMI, M., 1940. Fresh-Water Bryozoa of Manchoukuo. Rep. Limnobiol. Surv. Kwantung and Manchoukuo, conducted by Prof. T. Kawamura. pp. 425-432.
- KRAEPELIN, K., 1887. "Die Deutschen Süßwasser-Bryozoen."
- LEE, L., 1936. Notes on some Freshwater Polyzoa of Peiping. Sinensia, Vol. 7, pp. 399-407.
- ROGICK, M. D., 1934. Additions to North American Fresh-water Bryozoa. Ohio Jour. Sci. Vol. 34, No. 5, p. 316.
- , 1935. Studies on Fresh-water Bryozoa. II. The Bryozoa of Lake Erie. Trans. Micros. Soc. Vol. 54, No. 3, pp. 245-263.
- , 1937. Studies on Fresh-water Bryozoa. V. Some addition to Canadian Fauna. Ohio Jour. Sci. Vol. 37, No. 2, pp. 99-104.
- , 1940. Studies on Fresh-water Bryozoa IX. Additions to New York Bryozoa. Trans. Micros. Soc. Vol. LIX, No. 2, pp. 187-204.
- ROUSSELET, C. F., 1907. Zoological Results of the Third Tanganyika Expedition, conducted by Dr. W. A. Cunningham, 1904-1905. Proc. Zool. Soc. London. Vol. 1, pp. 250-257.
- VANGEL, E., 1894. Daten zur Bryozoen-Fauna Ungarns. Zool. Anz. 17, pp. 153-155.
- VORSTMAN, A. G., 1928. Some Fresh water Bryozoa from West Java. Treubia, Vol. 10, pp. 1-13.

EXPLANATION OF PLATE XIV

- Fig. 1. *Plumatella repens* var. *fuugosa* (PALLAS) from Mukden City. $\times \frac{1}{2}$
Fig. 2. *Plumatella repens* var. *annulata* HÔZAWA & TORIUMI from Mu-tan-k. $\times 2$
Fig. 3. *Hyalinella toanensis* HÔZAWA & TORIUMI from Mishanfu. $\times 2$
Fig. 4. *Plumatella* sp. from Dalai Nor. $\times 2$
Fig. 5. *Plumatella* sp. from Mishanfu. $\times 2$
Fig. 6. *Plumatella auricomis* ANNANDALE from Buir Nor. $\times 2$
Fig. 7. *Plumatella repens* var. *emarginata* (ALLMAN) from Dairen City. $\times 2$



TORIUMI photo.

S. HÔZAWA and M. TORIUMI: Freshwater Bryozoa of Manchoukuo.

THE CAVERNICOLOUS OLIGOCHAETA OF JAPAN, I

By

SHINRYÔ OHFUCHI

Zoological Division of Saitô Hô-on Kwai Museum, Sendai

(With 7 text-figures)

(Received November 28, 1940)

The purpose of this report is to describe a few species of the Cavernicolous Oligochaeta some already known to the scientific world, and some quite new. They have been collected during the past few years from various caverns in Kôchi, Ôita and Yamaguchi Prefectures, by Messrs. HAJIME TORII and JÛJIRÔ ISHIKAWA.

Previously to 1891, only two species of Oligochaeta from caverns were known, they were *Enchytraeus* (?) *cavicola* JOSEPH, and *Archaeodrilus cavaticus* CZERNIAVSKY. COGNETTI (1903) reported of a common species which he found that he noted no appreciable variations in the pigmentation. MICHAELSEN (1924 and 1926) described *Pelodrilus bureschi* from a cavern in Bulgaria, and it is interesting to learn that is earthworm was translucent white during life.

STEPHENSON (1924) describes five new interesting species of the genera *Enchytraeus*, *Dichogaster*, *Drawida*, and *Megascolides* from the Siju Cave in the Garo Hills of Assam, some of which are said to have been collected at a distance of 2,000 ft. from the entrance. All of these species are described as being pale in colour and unpigmented, and showing no difference between the dorsal and ventral surfaces.

Up to the present time there appears to be no detailed on the earthworms from caverns in Japan, thus the present report may be of some interest.

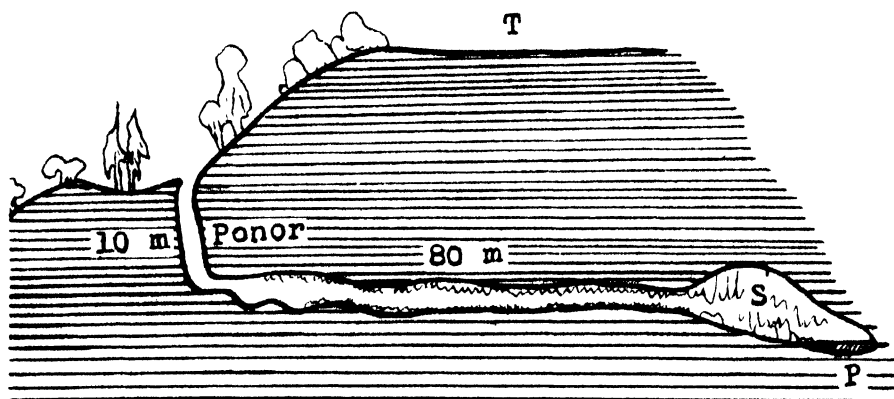
Before proceeding further, the writer wishes to express his hearty thanks to Messrs. HAJIME (元) TORII (鳥居) of Tokyo, and JÛJIRÔ (重治郎) ISHIKAWA (石川) Kôchi for supplying these interesting and important specimens and also for providing ecological data of the caverns.

He also wishes to express his hearty thanks to Prof. Dr. E. NOMURA for his helpful advice during the preparation of this paper.

1. THE SPECIES FROM THE CAVE OF SHÔNYÛDÔ SHINDÔ
(鐘乳洞新洞), ÔITA (大分)

Pheretima Toriii sp. nov.

Locality: Shônyûdô Shindô (鐘乳洞新洞), Fûren (風連), Tomari (泊), Kawanobori (川登) village, Ôno (大野) district, Ôita (大分) Prefecture. Specimen collected by Mr. HAJIME TORII, on August, 1st 1938, at a time when the specimens were mostly clitellated.



Text-fig. 1. Diagrammatic figure of the vertical section of the Shônyûdô Shindô cavern. P., Pool, S., Stalactite, T., Terrace.

Ecological conditions: The cavern, which is situated half way up a mountain, consists of two parts the entrance which is vertical (Ponor) and the main part which is nearly horizontal. The entrance is like a well of oblong shape, measuring about 1.5×0.6 m in diameter and is 10 m deep, while the main part of the cavern measures 80 m in total length. The worms in this cavern live in the surface of the soil which is moistened by water flowing in from the Ponor.

External characters:

The measurements of the body and number of segments of two individuals from Fûren Shônyûdô Shindô are as follows:

The body length varies between 37-43, (40 mm in average) the width at the male genital region of XVIII varies from 2.3 to 2.7 mm, (2.5 mm in average), and the number of segments varies from 77 to 83, (80 in average).

The colour shows no appreciable variations and no difference is noticed between the dorsal and ventral surfaces, except for the ventral surface

not being as pale as is usual in other specimens. The skin is relatively thick and stiff.

The spermathecal openings are in four pairs in the intersegmental furrows of V/VI, VI/VII, VII/VIII and VIII/IX, and are situated in the ventro-lateral margin.

The clitellum occupies the 3 segments, XIV, XV and XVI without setae, intersegmental furrows and dorsal pores.

The setae beginning on segment II. The setal numbers of the earthworms are tabulated below (Table 1).

TABLE 1.

Number of specimens	Segments				
	V	VI	VII	VIII	XX
1	35	40	41	43	56
2	33	39	42	45	58
average	34	39	41	44	57

The openings of the male pores are in segment XVIII and are situated close to the lateral border on the slightly elevated oblong area. The margins of the pores are considerably elevated over the body surface, gradually becoming more elevated and are surrounded by the 6 or 8 oblong-shaped rings, and are separated by 7 or 8 setae.

Female pore single midventrally on XIV.

The first functional dorsal pore is found in the intersegmental furrows XII/XIII.

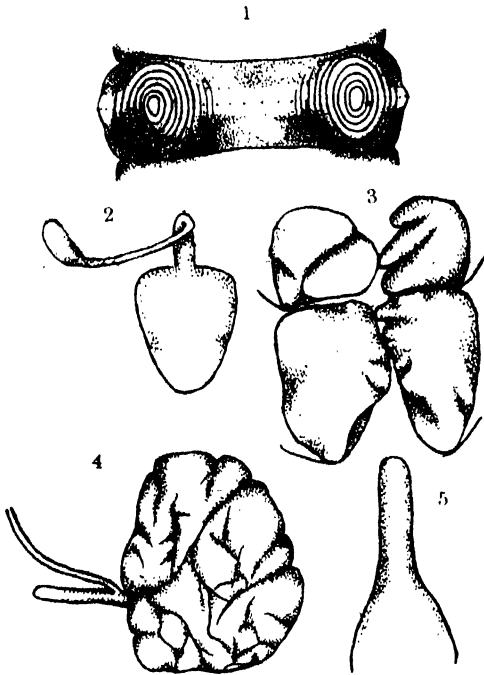
Genital papillae are not found in either the spermathecal and male pore regions.

Internal characters:

Spermathecae are situated in segments VI, VII, VIII, and IX.

The ampulla is cherry-shaped or spatulated and slightly dorsoventrally flattened; about 1.2 mm long, 1 mm broad in maximum and continuous to the spermathecal stalk which is about 0.5 mm long. The diverticulum is straight, much dilated at the tip, and forms a long chamber, this chamber reflects with metallic lustre, it opens in the terminal portion of the duct and is nearly equal to the combined length of duct and ampulla, being about 1.6 mm long.

Ovisac, one pair, it is small and hangs behind the septum XII/XIII.



Text-fig. 2. *Pheretima Toriui*, sp. nov. 1. Showing the ventral view of the male pores of the segment XVIII, $\times 14$. 2. Spermatheca of the right side, $\times 14$. 3. Showing the structure of the seminal vesicle in dorsal view. 4. Showing the shape of the prostate glands with the duct, $\times 14$. 5. Showing the shape of the intestinal caeca in lateral view from the left side, $\times 14$.

XII/XIII, XIII/XIV and XIV/XV more or less thickened, XI/XII and XII/XIII rather thickened.

The seminal vesicles barely meeting middorsally, posterior pair slightly larger than the anterior. Testis sacs in X and XI, rather large.

Remarks:

This species is the smallest among those possessing four pairs of spermathecae in the genus *Pheretima* from Japan.

No appreciable variations in the colour or the pigmentation were observed among the specimens living in the cavern, and no difference was noticed between the dorsal and ventral surfaces. The skin of this species is rather thick and stiff.

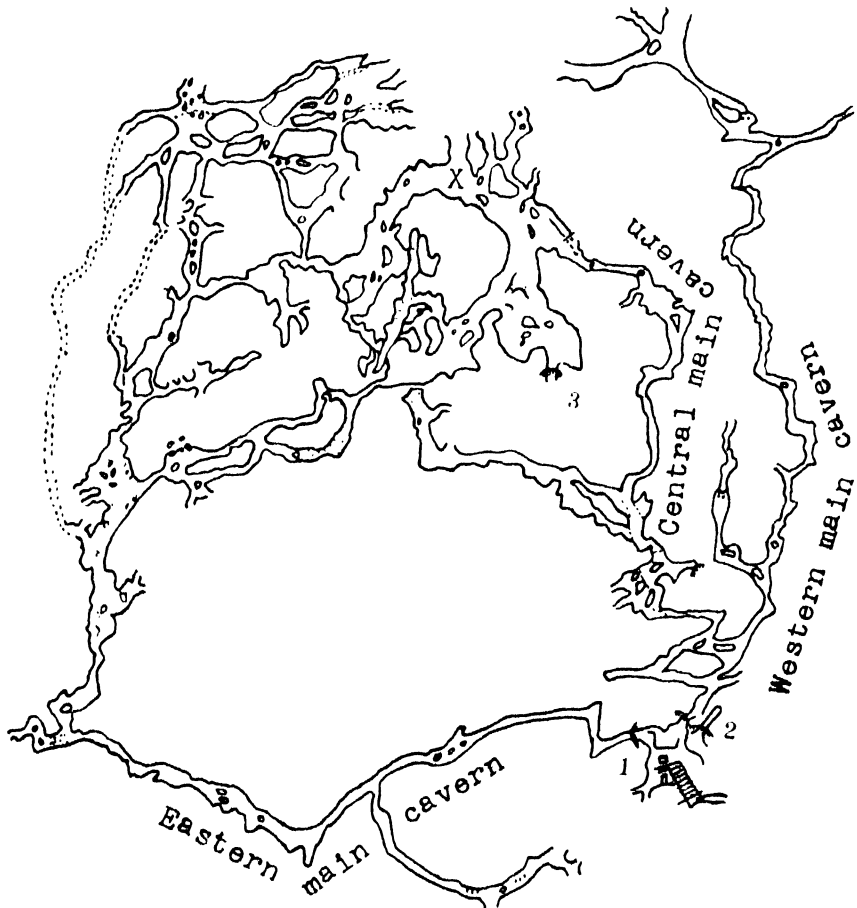
Prostate gland well-developed, rather thin, about 2.3 mm long, 1.4 mm wide, each divided into three main lobes, and extending from XVI-XX (5 segments); muscular duct not bending and not as muscular as is usually seen in the other species of the genus, rather thin and soft, not reflecting the lustre of the tension muscle which forms the duct as is usually seen in many other species of the genus.

The saccular intestine originates in XIV and a pair of the intestinal caeca begin in XXVII and extend to XXII anteriorly. The caeca is a single and conical projection, without serriformed projection in both ventral and dorsal margins, nearly amooth, with slight septal constrictions.

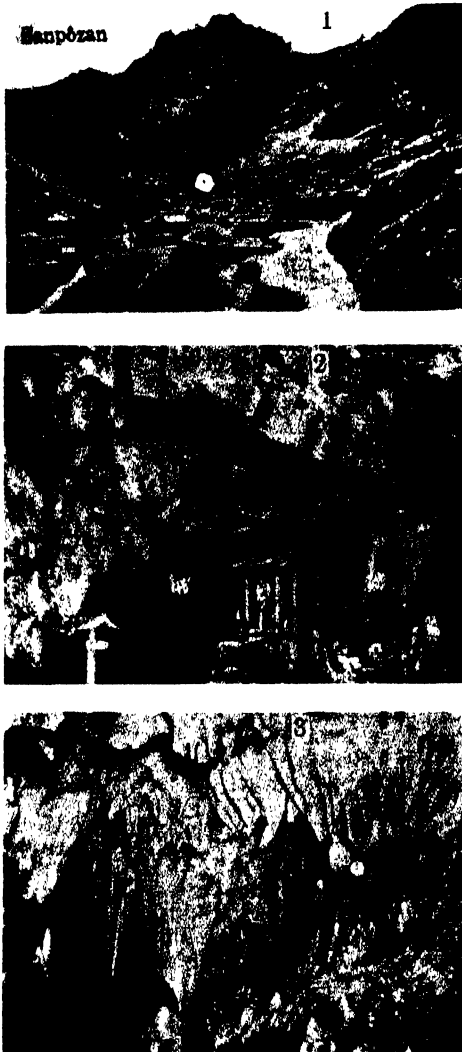
Septa generally very thin VI/VII and VII/VIII more or less thickened, VIII/IX, and IX/X lacking, X/XI, XI/XII,

The muscular duct of the prostate gland in this species does not bend as usually seen in the other species of this genus. Presumably this species merely wandered into the caves from near by regions, or it probably was carried in with the soil from the Ponor. But in the absence of further knowledge of the Oligochaeta fauna of the exterior of this cave no conclusions can be given here.

2. THE SPECIES FROM THE CAVE OF RYÜGADÔ (龍河洞), KÔCHI (高知)



Text-fig. 3. A plane figure of the Ryûgadô cavern. 1. Entrance to the eastern main cavern. 2. Entrance to the western and central main caverns. 3. Entrance faces north in the direction of the south-eastern side, and its floor is about 80 m higher than that of the other two entrances, and is the exit from the cavern. X., Showing the collected place of the new species *Pheretima Ishikawai*, about 160 m from the entrance of the eastern main cavern.



Text-fig. 4. 1. Showing the entrance of the Ryûgadô cavern. (White circular mark). 2. Showing the entrance of the Ryûgadô cavern. 3. Showing a part of the interior of the Ryûgadô cavern, the place is named "Banshōden" (萬象殿). All according to a photograph sent to the present writer by Mr. JŪJIRŌ ISHIKAWA.

Pheretima Ishikawai sp. nov.

Locality: Ryûgadô, in Mt. Sampôzan (三寶山), Kami (香美) district, Kôchi (高知) Prefecture. About 20 km, east of Kôchi.

This species was collected in June, 1939, by Mr. JŪJIRŌ ISHIKAWA, at a time when the specimens mostly clitellated.

Ecological conditions: Ryûgadô is a limestone cavern situated half way up Mt. Sampôzan. This cavern extends in 4 km total length underground, but the length of the main cavern is only 1 km, and it has three principal entrances, one in the direction of the western side from the foot of Sampôzan, which is the entrance to the eastern main cavern; the second is on the opposite side to the entrance of this eastern main cavern, and is the entrance to the western and central main caverns; and the third entrance faces the north in the direction of the south-eastern side, and its floor is about 80 m higher than that of the other two entrances, and is the exit from the cavern. About 420 m of the total length of the cavern is filled with subterranean water, which is the source of the Sakagawa (逆川) river. The limestone formation of Sampôzan is said to belong to the Triassic period, although there seems to

be no Palaeontological evidence

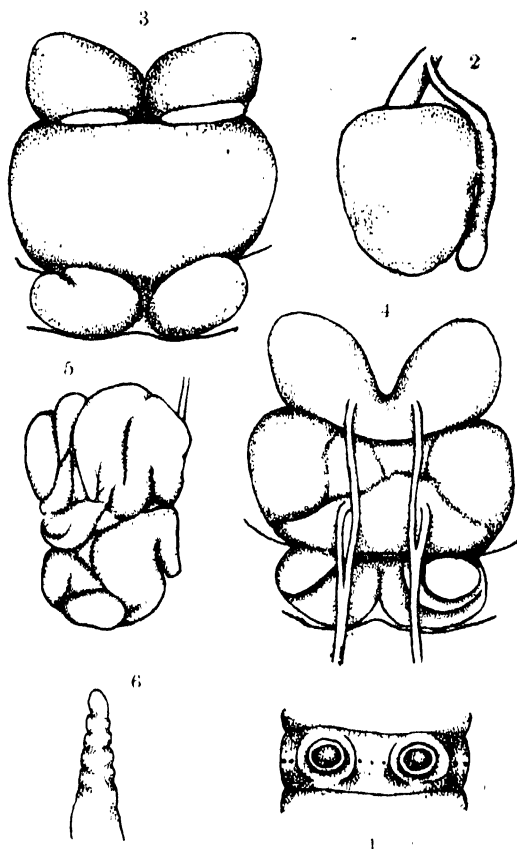
This species is found living at about 160 m from the entrance of the eastern main cavern. The temperature at this spot, although at such a distance from the entrance is affected by the atmosphere of the outside of the cavern. The place most suitable for this new species seems to be in the accumulated deposits of dung of *Phinolophus ferrumequinum nippon* TEMMINCK. According to Mr. HAJIME TORII the air temperature was 15°C. and the humidity was 82° from July 27th to 29th, 1938. This place is very suitable for this new species.

External characters:

The measurements of the body and number of the segments of three individuals from Ryûgadô are as follows:

The body length varies between 47–65 mm, (56 in average) the width at the male genital region of XVIII varies from 2.3 to 2.8 mm, (2.5 mm in average), the number of segments varies from 60 to 72, (64 in average).

The colour is pale and unpigmented, and no difference was noticed between the dorsal and ventral surfaces. The skin is rather thin so that the dorsal blood-vessels in this species can be seen very easily through



Text-fig. 5. *Pheretima Ishikawai* sp. nov. 1. Showing the male pores of the segment XVIII in ventral view, $\times 14$. 2. Spermatheca, left side $\times 14$. 3. Showing the structure of the seminal vesicle from the dorsal side. 4. The seminal vesicle from the ventral side. 5. Showing the structure of the prostate glands with the duct, $\times 14$. 6. Showing the structure of intestinal caeca in lateral view from the left side, $\times 14$.

the semitransparent integument, especially in the very well developed seminal vesicles. This character is worthy of specific value.

The spermathecal openings consist of one pair in the intersegmental furrow V/VI, and are situated in the ventro-lateral. The pores are small.

Clitellum occupies 3 segments, XIV, XV and XVI without setae, intersegmental furrows and dorsal pores.

Setae beginning on segment II. Setal numbers of the earthworms are tabulated below (Table 2).

TABLE 2.

Number of specimens	Segments				
	V	VI	VII	VIII	XX
1	39	39	40	40	45
2	37	39	40	41	44
average	38	39	40	41	45

The openings of the male pores are in segment XVIII and are situated close to the lateral border on the slightly elevated oblong area. The margins of the pores are considerably elevated over the body surface, and are separated by 1 or 2 setae.

The first functional dorsal pore is found in XII/XIII.

The seminal vesicles are very well developed, on account of this character the segments X, XI, XII are much distended on the outside.

Genital papillae are not found in either the spermathecal and male pore regions.

Internal characters:

One pair of spermathecae are situated in segment VI. The ampulla is ovoid and is a little flattened dorso-ventrally; about 1.7 mm long, 1.3 mm broad in the widest part and continuous to the spermathecal duct which is about 1/2 the length of the ampulla. The diverticulum is slender and nearly equal to the combined length of the duct and ampulla, being about 2.3 mm long, not coiled or looped; it is swollen at the end and forms a round chamber.

Ovisac, one pair, it is small and hangs behind the septum XII/XIII.

Prostate gland about 2.3 mm long, and extends through XV-XXI (about 7 segments occupied). The muscular duct does not bend as is usually seen in the other species, open to segment XVIII, straight from

the prostate gland, in this way, the muscular duct hides itself under the gland.

The saccular intestine originates in XVI and a pair of the intestinal caeca originates in XXVII and extends to XXIV anteriorly. The caeca is a single, conical projection, serriformed in both ventral and dorsal margins, and with slight septal constrictions. Septum VI/VII and VII/VIII more or less thickened, VIII/IX and IX/X absent, X/XI, XI/XII, XII/XIII, XIII/XIV and XIV/XV thickened, XV/XVI more or less thickened.

The seminal vesicles are well developed and are situated in XI and XII, very large, each anterior vesicle of this species is completely combined at both the dorsal and ventral sides and forms a doughnut-shape with the vesicle. Testis sacs in X and XI, anterior pair rounded, and projecting into X, connected in ventral medially with its opposite by a bridge; testis round in shape situated at the anterior inner wall of each sac. Spermduct united in XII.

Remarks:

It is of considerable interest to find that the present species possesses one pair of spermathecae. BEDDARD (1895), recorded in his monograph nine species which possess only one pair of spermathecae in the genus *Pheretima*. Among them only one species, *Pheretima pusilla* (UDE) collected from Buitenzorg and Java, has the one pair situated in segment VI, while the others all possess one pair of spermathecae in each of the segments V, VII, VIII and IX.

MICHAELSEN (1900) recorded 26 species of worms in his "Das Tierreich", and particularly five species, viz., *Pheretima phacellotheca* (MICHAELSEN), *Pheretima biserialis* (E. PERRIER), *Pheretima lompobatangensis* (MICHAELSEN), *Pheretima pusilla* (UDE) and *Pheretima urceolata* (HORST) which are said to possess one pair of spermathecae in segment VI.

Only one species, *Pheretima schizopora* reported by GOTÔ and HATAI (1898) from Tokyo, Japan, has the spermathecae situated in segment VIII, that of the left side is said to have three diverticula, and that of the right side only one. This species appears to differ in the position of the spermatheca, which is one of the most important characters of the species.

The spermathecae in the former are situated in VIII, instead of in VI as in present one. Such a species with one pair of spermathecae in segment VI has hitherto been unrecorded in Japan. It was collected from the Ryûgadô cave and is pale in colour and unpigmented, showing no difference between the dorsal and ventral surfaces; such characters are not found in the common species living in outer ordinary conditions,

as for instance in those uncavernicolous Oligochaeta as *Pheretima divergens* (MICHAELSEN) and *Pheretima masatacae* (BEDDARD) which have never been collected from the Ryûgadô cave.

Undoubtedly the present new species here recorded can be regarded as a true cavernicolous earthworm.

***Pheretima divergens* (MICHAELSEN)**

1892. *Perichaeta d.* MICHAELSEN in: Arch. Nat., v. 581, p. 234, t. 13, f. 21.

1899. *Amyntas d.*, MICHAELSEN in: Mt. Mus. Hamburg, v. 16, p. 8.

The specimen collected by JÛJIRÔ ISHIKAWA on August, 1939, near the entrance of the cave is fully mature sexually. Probably this specimen merely wandered into the cave from the outside as there are no noticeable morphological differences between it and the same species living in ordinary conditions, thus it not a true cavernicolous form.

***Pheretima masatacae* (BEDDARD)**

1892. *Perichaeta masatacae*, BEDDARD in: Zool. Jahr. Syst. Geogr. Biol., Bd. 6, pp. 761-762.

This specimen collected by JÛJIRÔ ISHIKAWA on August, 1939, near the entrance of the cave, is fully mature in both external and internal features, the sex organs and genital markings. This specimen shows no morphological differences from the ones existing under normal conditions outside the cavern.

***Pheretima sakaguchii* OHFUCHI**

1938. *Pheretima sakaguchii*, OHFUCHI in: Res. Bull. No. 15, pp. 53-58.

This specimen collected by HAJIME TORII on July, 1938, in the inner part of the cave, is half mature sexually, showing no morphological differences from those living under normal conditions.

***Pheretima* sp. juv.**

This immature specimen collected by HAJIME TORII on July, 30th, 1938 from the inner part of the cave, is too juvenile for specific identification.

3. TREE SPECIES FROM THE CAVE OF SHŪHÔDÔ
(秋芳洞), YAMAGUCHI (山口)

Pheretima sp. juv.

Local y: Shūhōdō, Akivoshi (秋吉), Mine (美禰) district, Yamaguchi Prefecture.

The species was collected on August 29th, 1938, by Mr. HAJIME TORII, at a time when the specimens were acitellated and the genital organs and their marking not yet mature. Thus the identification of the species was impossible.

Ecological conditions: Shūhōdō is a limestone cavern the entrance of which is at the eastern foot of a precipice about 50 m south of Shūhōdai (秋芳臺). There is subterranean water flowing out of the cave. The principal chamber of this cave is 2 km long, 90 m. in maximum width, and 10 m in height.

The air temperature was 15.5 C. and the humidity was 83% from the 21th of August to the 3rd September, 1938, in this cavern, according to Mr. HAJIME TORII.

This species is said to be found at a spot about fifty or sixty meters from the entrance of the cavern, at a place where the Yoi-no Myōjō (宵の明星) (Doline) is connected with the cavern.

The soil of this place is rich in vegetable organic matters and air-slaked soil, which is probably carried in with the rain water from the Shūhōdai. Probably this specimen also was carried into the cavern since no morphological differences were noticed between it and the ones living under normal condition.

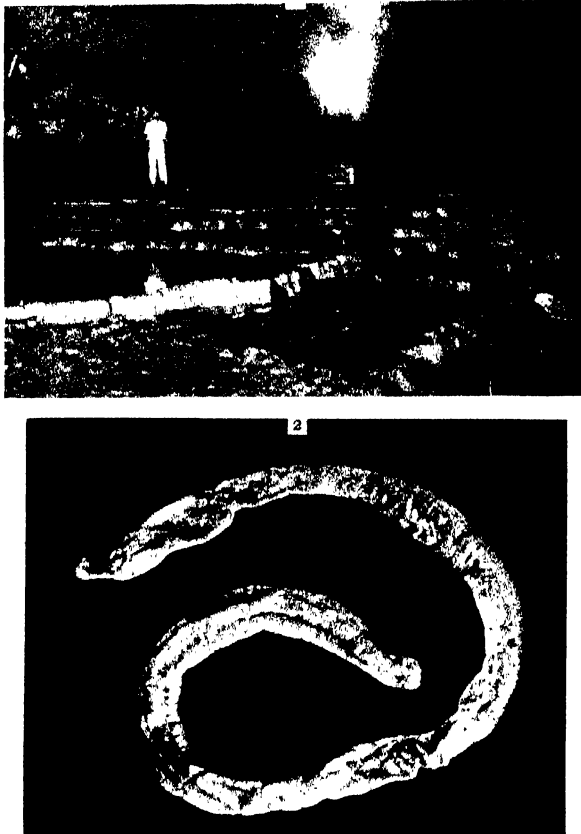


Text-fig. 6. Entrance of the Shūhōdō cavern; photographed by Mr. HAJIME TORII

Drawida sp.?

Habitate: This worm is found living in the mud of the so-called

“Senchôda” (千町田), which is a name given to a shallow variously shaped pools formed and separate by the precipitate of calcareous sinter, about 1 km from the entrance of the Shûhodô cavern. The material consisted of only a single specimen, thus its specific identification was not possible owing to poor fixation, therefore on this occasion only its photograph



Text-fig. 7. 1 The so-called “Senchôda” which is formed by the precipitate of calcareous sinter. Many pools filled with clear water are formed the Senchôda being situated about 1 km from the entrance of the cavern. 2. This worm was collected on August 9th 1938 from the mud of Senchôda; it is perfectly translucent white, $\times 3$

will be published. Detail accounts will be given when more specimens accumulate.

The water temperature of this pool was 15.5°C . when the air temperature was 17.8°C . and the pH was 7.0 on the August 30th 1938. The air

temperature was 15.5°C. and humidity 83° from the August 24th to September 3rd 1938, according to Mr. HAJIME TORII as already stated.

Lumbriculus sp.

Habitate: This worm is found living in the Senchôda. Judging from the external structure, it is clear that this specimen belongs to the genus *Lumbriculus*, although the specific name was not determined.

CONSIDERATION

The results of observations on cavernicolous Oligochaeta collected from various caverns of Japan, are as follows.

1. The Oligochaeta may be generally divided roughly into three groups by their dwelling habits, viz. the earth-dwellers, the mud-dwellers and water-dwellers. The cavernicolous Oligochaeta possess the combined characters of these three groups, because the caverns, being isolated from the outside, are under the influence of conditions quite different from those outside, viz. the degree of moisture, food, temperature, nature of the soil, light, and other conditions, biological, chemical, physical.

2. Earthworms are sensitive to the presence of water, but has been proved that some can endure submersion in water for a long time. COMBAULT (1909) has kept worms in clear water for several months, though he admits that they perish in the soil when it is too damp, but doubtless that is because the compact soil does not permit renewal of the respiratory air. The writer (1938) kept *Allolobophora foetida* (SAVIGNY) alive for nearly three months completely submerged in water. On earthworms, both temperature and hydrated soil have a considerable influence.

3. The earthworms living in caverns, in which they have been isolated for long periods, and in which they are confined with no migration to the outside, show features different to those of the normal worms. These differences due to the effect of considerably different conditions, inside and outside the caverns, are well displayed in the specimens, and a good example is the lack of pigment in the cavern-dwellers which is due to living in the dark. In the specimens which migrate freely to and from the cavern, no appreciable difference is noticed in the pigment.

4. Even in July or August, when the worms living under normal conditions usually reach full maturity both externally and internally in the sex organs and in the genital markings, many of the specimens

which are found in the caverns are still immature, remaining in a young stage. Presumably these cavernicolous specimens have not reached maturity owing to their not receiving as much light as those which exist outside the cavern. It is noteworthy that the air temperature in the caverns is almost the same throughout the four seasons of the year. Therefore, it seems that the period of the younger stage is considerably lengthened, and that they hibernate in this stage.

5. Owing to insufficiency of food and the other factors mentioned above in the cavern, the size of the specimens is very small in general and this is a specific character of the cavernicolous species.

REFERENCES

- BEDDARD, E. E., 1892. On some Perichaetidae from Japan. Zool. Jahr. Bd. 6, Heft, 5.
 COGNETTI, L., 1903. Contributo alla conoscenza degli Oligocheti cavernicoli. Atti Soc. Nat. Modene, (4), 5.
 —, 1904. Nota su alcuni Lombricidi di caverne italiane. Boll. Mus. Torino, 19.
 —, 1904. Descrizione di un nuovo Lombrico cavernicolo. Boll. Mus. Torino, 19.
 —, 1927. Lombricidi dei Carpazi, Boll. Mus. Genova, (2), 7.
 COMBAULT, A., 1909. Contribution à l'étude de la respiration et de la circulation des Lombriciens. J. Anat. Paris, 45.
 GOTÔ, S. and HATAI, S., 1898. New or Imperfectly known species of Earthworms. Ann. Zool. Jap., Vol. II.
 —, 1899. New or Imperfectly known Species of Earthworms. Ann. Zool. Jap., Vol. III.
 LENGERSDORF, E., 1926. Beitrag zur Höhlenfauna des Siebengebirges. Sitz-Ber. natur. Ver. Preuss. Rheinl. Westf. (Bonn).
 MICHAELSEN, W., 1892. Terricolen des Verliner Zoologischen Sammlung, II, Arch. f. Nat., Lvii, Pl. Xiii.
 —, 1899. Terricolen von Verschiedenen Gebieten der Erde. Mitt. Nat. Mus. XVI. (Jahrb. Ham. Wiss. Anst. XVI).
 —, 1924. Ein Süßwasser-Höhlenoligochät aus Bulgarien, Mitt. Zool. Hamberg. 41.
 —, 1924. Oligochäten von den Würmeren Gebieten Amerikas und des Atlantischen Oceans, Mitt. Zool. Mus. Hamberg 41.
 OHFUCHI S., 1938. 石狩沃野の水田に發生する蚯蚓 *Pheretima* 屬に對する動物學的考察. 植物及動物. 第6卷. 第12號. (Zoological Observation on the Earthworms of the Genus *Pheretima* from the Rice Field in Ishikari, Hokkaidô. Bot. Zool. Vol. 6, No. 12.
 PICKFORD, G. E., 1938. VI Earthworms in Yucatan Caves. Carnegie Washington Pub. No. 491.
 STEPHENSON, J., 1924. The Siju Cave, Garo Hills, Assam: Zool. Hahr. Bd. 6, Heft 5.

AN OUTLINE OF THE DEVELOPMENT OF *CYNTHIA RORETZI* DRASCHE

By

ETURÔ HIRAI

Biological Institute, Tôhoku Imperial University, Sendai

(With Plates XV XVI)

(Received December 14, 1940)

The development of the Ascidian egg has long been studied by many authorities. The sea-squirt, *Cynthia roretzi* DRASCHE, is abundant on the Pacific coast of the northern provinces of Japan and is cultivated in some places. During January 1940, the writer had the opportunity to catch the outline of the development of the sea-squirt by way of a series of photomicrographs. In the present paper, the writer will give briefly an embryological observation with reference to these serial photomicrographs.

Before proceeding further, the writer wishes to express his great thanks to Prof. Dr. E. NOMURA¹⁾, under whose direction and encouragement the present study has progressed. The writer is also greatly indebted to Dr. I. MOTOMURA and Mr. K. OKADA for their valuable advice during the course of this study.

MATERIAL AND METHOD

The material used in this study was supplied to the laboratory in Sendai, it was obtained from HATAKEYAMA's culture-station of the sea-squirt at Hinatagai, Kesenuma, Miyagi Prefecture, and from a locality near the Onagawa Oceano-chemical Laboratory. The material lived for a day kept in an ice-box at 7°C in the laboratory. The gonads were then cut out from the body of the animal into Petri dishes containing seawater. The eggs and the sperms lived for about twelve hours in the removed gonads. To take out the living eggs and sperms from the gonad, the walls of the oviduct and the sperm-duct were punctured with fine scissors and the germ cells sucked out with a pipette into the dishes.

¹⁾ It is, here, worthy of note that the present investigation was carried out at the expense of Kagakukankyuhi from the Department of Education, Imperial Japanese Government.

The eggs were artificially inseminated in artificial seawater according to Herbst (1904) which was diluted to the salinity of the Pacific coast at Matusima. The fertilized eggs were cultured in the Petri dishes at 13-14°C in the thermostat. The present observation was carried out on these cultured eggs.

RESULTS OF OBSERVATIONS

The egg contains yellow yolk granules, and is enveloped in transparent membrane (Pl. XV, Fig. 1). The mature egg, including the chorion and the follicular epithelia, measures about 360 μ in diameter, and the primary oöcyte itself measures about 270 μ . A large, transparent germinal vesicle is seen near a pole of the unfertilized egg. About thirty minutes after, the germinal vesicle gradually disappears, and an hour after it diminishes to a transparent spot near the pole of the egg. After about four hours, the first polar body is extruded, and then the second polar body is formed about twenty minutes after the extrusion of the first polar body (Pl. XV, Figs. 2 & 3). Following the disappearance of the germinal vesicle, the test-cells embedded in the peripheral cytoplasm of the oöcyte are expelled from the cytoplasm. At the same time the perivitelline-space appears. The perivitelline-space increases gradually, until the diameter of the envelope becomes twice as long as that of the oöcyte itself. The test-cells become free in the space, and the development of the egg progresses in it (Pl. XV, Figs. 2-12, & Pl. XVI, Figs. 13-21). The deformation of the egg shape occurs in the uncleaved stage (HIRAI, 1941).

E. G. CONKLIN (1905) reports with regard to the egg of *Cynthia partita*, that cytoplasmic movements of a violent character take place while the maturation divisions are going on. In this case the clear substance has the yellow pigment granules at the peripheral layer. The same phenomena take place also in *Cynthia roretzi*, but there is no pigment in the egg-plasm of this form, as stated in a previous paper (HIRAI, 1941).

The distribution of the yolk in the immature egg shows a concentric structure. The germ formation is holoblastic. The cleavage type is bilateral. The first division, a meridional one, divides the egg into two equal halves (Pl. XV, Fig. 4). The direction of the first division plane indicates the median plane of the embryo. The second division is meridional and the plane is at right angles to the first cleavage plane (Pl. XV, Fig. 5). In this four-cell stage, it is difficult to recognise the inequality of blastomeres. The third division is somewhat unequal and is latitudinal,

resulting in four cells of the animal-half which are smaller than the corresponding ones of the vegetative half. The inequality of blastomeres between the animal and vegetative halves is clearly shown in Pl. XV, Fig. 6. The fourth division is not as strictly meridional as might be expected. The division plane of the anterior two blastomeres at the animal side intersects with the first division plane, and that of the two posterior cells intersects with the second division plane (Pl. XV, Fig. 7). In the vegetative half the converse relations hold (Pl. XV, Fig. 8). A difference of the external outlines in the same cleavage stage may be caused by the cleavage cycle of the blastomeres, that is, just after the completion of a division the blastomeres showed spherical forms as shown in Pl. XV, Fig. 7, and then they came in close contact as shown in Pl. XVII, Fig. 8. The next cleavage period raises the number of cells to thirty-two (Pl. XV, Fig. 9). When the egg is at about the sixty-four-cell stage, it becomes blastula (Pl. XV, Fig. 10). The wall of the blastula is very thick, and therefore, the segmentation cavity is not recognizable in the living blastula. The blastula has no cilia. The blastula is about 220μ in length, and about 300μ in width. The later blastula becomes flattened a little in the dorso-ventral direction. At the stage of from one hundred and twenty-four to two hundred and forty-eight cells, the blastula grows to a gastrula (Pl. XV, Figs. 11 & 12, and Pl. XVI, Fig. 13). As gastrulation proceeds, a dish-shaped gastrula changes into a bowl-shape (Pl. XVI, Figs. 14, 15 & 16), and then it becomes a young tadpole, showing the formation of the neural plate and the neural folds (Pl. XVI, Figs. 17 & 18).

The posterior part of the embryo is elongated, and the tail of the tadpole is formed there (Pl. XVI, Figs. 19, 20 & 21). The embryo in the stage of Pl. XVI, Fig. 21 begins convulsionary movements at intervals in the follicular membrane. At this stage it is difficult to recognize externally the sensory organs. When the fixing process is formed at the anterior end of the body, the two black sensory organs become distinct in the antero-ventral portion of the body. At this stage, the tadpole shows sometimes violent movements. The fully developed larva, which is about 1.5 mm. in total length, hatches out of the enveloping membrane. Its body measures about 330μ in length, and about 200μ in width. The tail is about 1.2 mm. in length and 80μ in width. The tadpole has no pigment cells except the yellow yolk, and is comparatively transparent in the external view. The total surface of the tadpole is covered with a thin, gelatinous cuticle (Pl. XVI, Fig. 22). The tadpole enters on its

free-swimming life. About twenty-four hours after hatching, the tadpoles stop their movements on the bottom of the Petri dish, but none of the tadpoles attached completely to it was obtained. The settled tadpoles undergo a rapid degenerative metamorphosis (Pl. XVI, Figs. 23 & 24). By this metamorphosis the body becomes round and chin region grows enormously (Pl. XVI, Fig. 23). Although the time-span necessary for the metamorphosis was not observed, the writer supposes that it is completed within one or two hours. Whilst the metamorphosis is going on, granular particles become visible in the gelatinous cuticle enveloping the embryo (Pl. XVI, Figs. 23 & 24).

PUBLICATIONS REFERRED TO

- CONKLIN, E. G. 1905. Organization and cell-lineage of the Ascidian egg. *Vide* COWDRY and other text-books.
 COWDRY, E. V. 1924. General Cytology. Chicago.
 HIRAI, E. 1941. The Early Development of *Cynthia roretzi* DRASCHE. Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. XVI.

EXPLANATION OF PLATE ($\times 90$)

The plane of bilateral symmetry in these developing eggs or embryos is perpendicular to the plane of the plate in Figs. 4-12, 14, 15, 17, 19 and 20, and is parallel in Figs. 13, 16, 18, 21 and 22.

PLATE XV.

- Fig. 1. An unfertilized egg immediately after being shed into seawater. Transparent membranes are shown around the oöcyte, in which the germinal vesicle is visible as a somewhat light area, and test-cells are not shown.
 Fig. 2. A developing egg four hours later. Two polar bodies had already extruded. A cytoplasmic process at the animal pole is one of the changes in shape of the oöcyte. The flow of cytoplasmic substance is shown by the clear part at the vegetative pole. The envelope, constituted of chorion and follicular epithelium, is swelling and the test-cells become free in the perivitelline space.
 Fig. 3. A lateral view of developing egg a little after the stage shown in Fig. 2. The egg returned to a spherical form. The light part has moved to the posterior side of the egg.
 Fig. 4. A posterior view of two-cells stage (about five hours later).
 Fig. 5. A polar view of four-cell stage (about five and a half hours later).
 Fig. 6. A polar view inclining a little to the posterior side of eight-cell stage. The inequality of the blastomeres is shown between the animal and the vegetative side.
 Fig. 7. A polar view of sixteen-cell stage.
 Fig. 8. An antipolar view of sixteen-cell stage.
 Fig. 9. An antipolar view of thirty-two-cell stage.
 Fig. 10. A polar view of blastula.

Fig. 11. An antipolar view of gastrula (about seventeen hours after).

Fig. 12. A polar view of early gastrula.

PLATE XVI.

Fig. 13. A left side view of early gastrula. The external shape of the gastrula is shown as a dish-form.

Fig. 14. An antipolar view of later gastrula.

Fig. 15. A polar view of later gastrula.

Fig. 16. A side view of later gastrula.

Fig. 17. A dorsal view of young tadpole, showing the formation of neural plate and neural folds.

Fig. 18. A right side view of young tadpole.

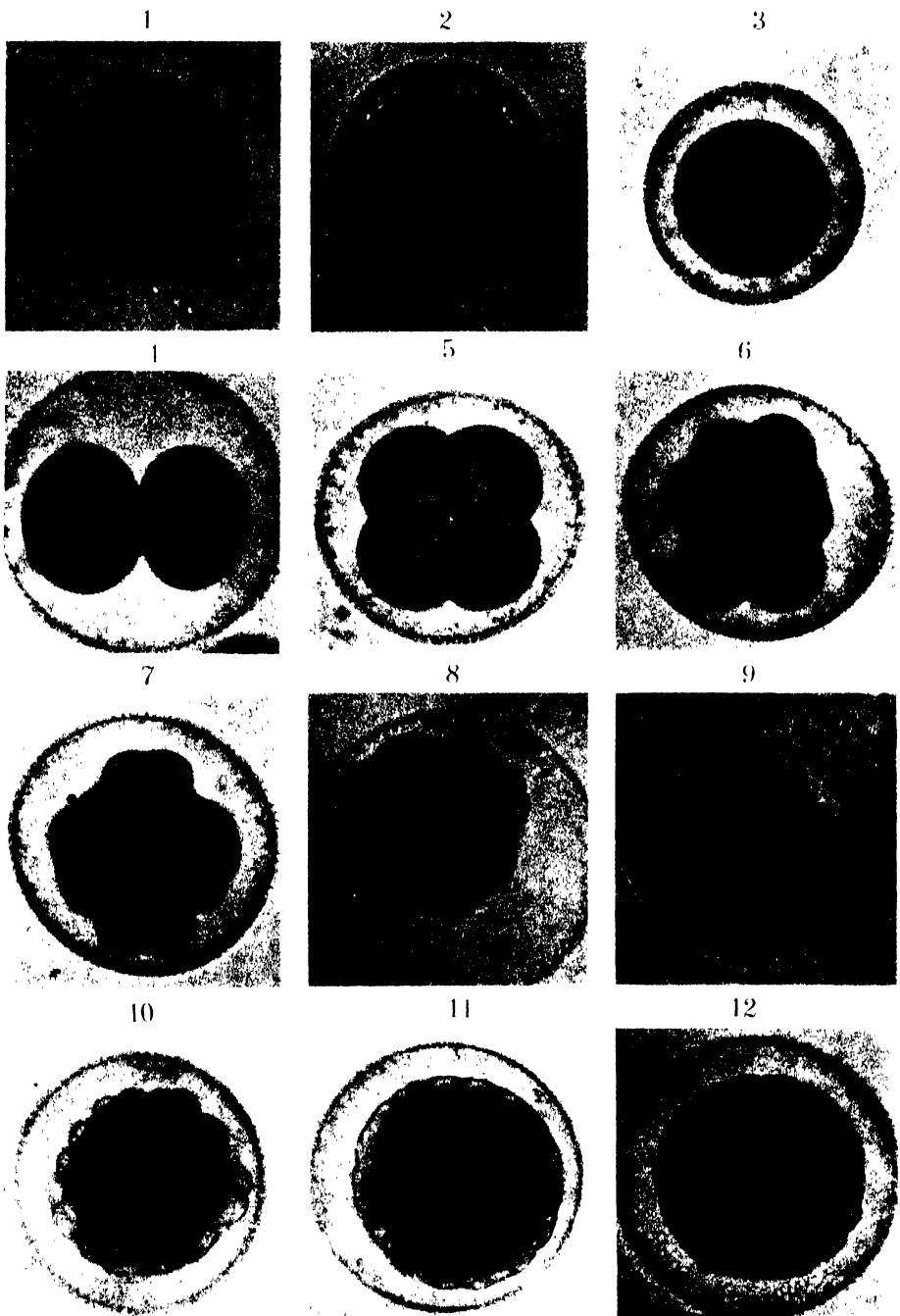
Fig. 19. A frontal view of advanced young tadpole (about twenty-one hours later).

Figs. 20 and 21. More advanced stages of young tadpole. The tadpole of these stages showed a convulsionary movement.

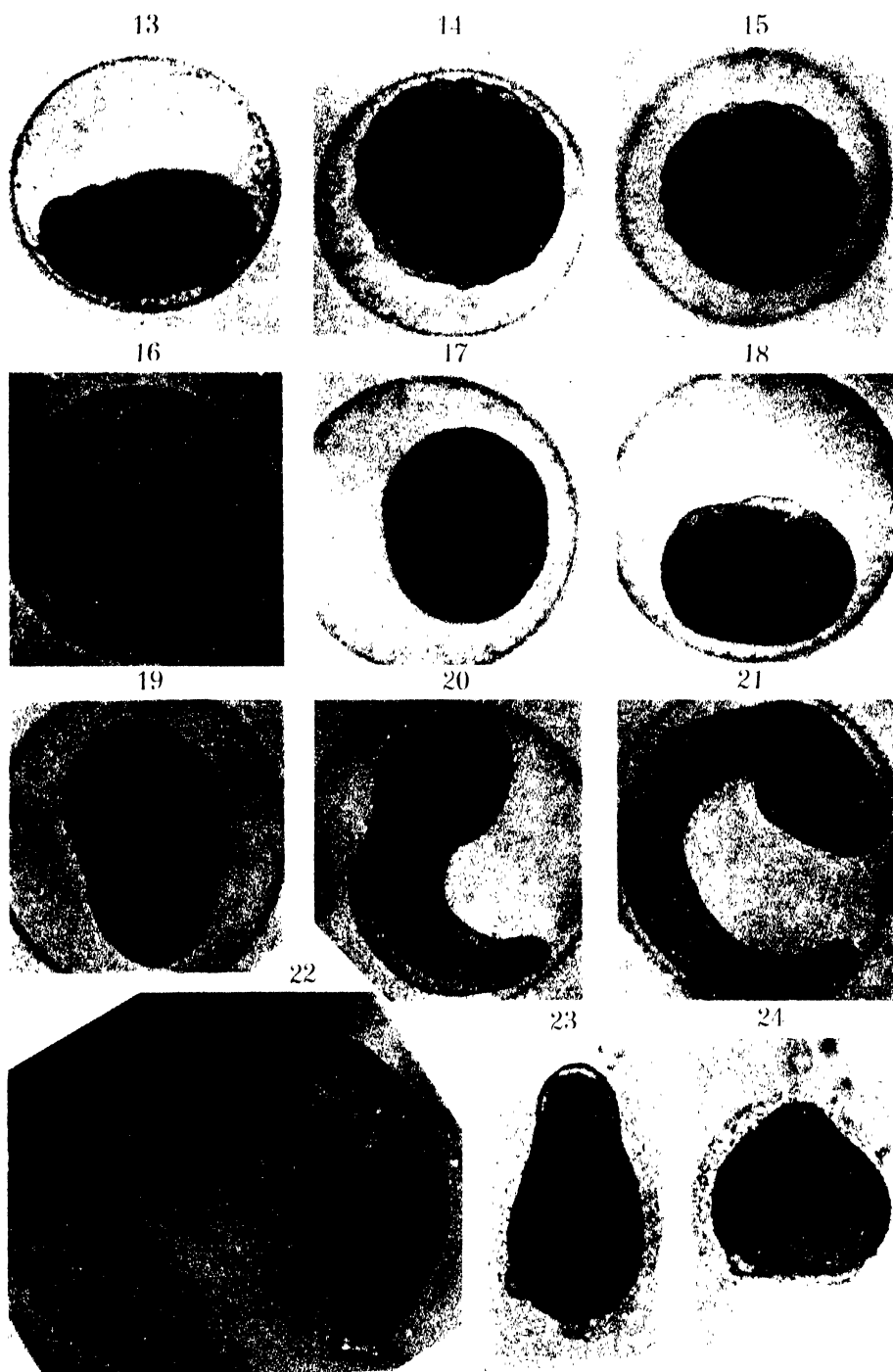
Fig. 22. A free-swimming tadpole, 1.5 mm. long (about forty-six hours later). The total body is covered with gelatinous cuticle. Sensory organs are shown at the anterior part of the body.

Fig. 23. A stage of the degenerative metamorphosis of tadpole. The tail is degenerating, the body became spherical, the chin region grew enormously.

Fig. 24. A more advanced stage of the metamorphosis of the tadpole (about seventy-five hours later). In the last two stages shown in Figs. 23 and 24, granular particles appeared in the gelatinous cuticle.



HIRAI photo



HIRAI photo.

CALCAREOUS SPONGES OBTAINED FROM ONAGAWA BAY AND ITS VICINITY

By

SENJI TANITA

Biological Institute, Tôhoku Imperial University, Sendai, Japan

(With Plate XVII and 4 Text-figures)

(Received January 15, 1941)

The material of the calcareous sponges dealt with in the present study was chiefly secured by the writer from Onagawa Bay and its Vicinity during his short stay at Onagawa in July, 1939, and in June, 1940. Some specimens were obtained by Professor HOZAWA in August, 1940, and the several others were collected by Dr. T. IMAI of the Onagawa Oceano-Chemical Institute of the Tôhoku Imperial University in May, 1935, from the same vicinity. Most of the specimens above alluded to are preserved in the museum of the Biological Institute of the Tôhoku Imperial University in Sendai.

They are referable to the eighteen species listed below, of which three are now described for the first time.

Family Homocoelidae

1. *Leucosolenia canariensis* (MICHLUCHO-MACLAY)
2. *Leucosolenia laxa* KIRK
3. *Leucosolenia mollis*, n. sp.
4. *Leucosolenia mutsu* HOZAWA
5. *Leucosolenia tenera* TANITA

Family Sycettidae

6. *Sycon ciliatum* (FABRICIUS)
7. *Sycon lendenfeldi* ROW and HOZAWA
8. *Sycon mundulum* LAMBE
9. *Sycon okadai* HOZAWA
10. *Sycon ornatum* KIRK
11. *Sycon rotundum*, n. sp.
12. *Sycon simushirensis* HOZAWA

Family Heteropiidae

13. *Vosmaeropsis maculata* HOZAWA

Family Grantiidae

14. *Leucandra abratisbo* HOZAWA
15. *Leucandra mediocanellata* HOZAWA
16. *Leucandra tomentosa* TANITA
17. *Leucandra valida* LAMBE
18. *Leucandra vermiformis*, n. sp.

Before proceeding further, the writer wishes to express his sincere thanks to Professor HOZAWA for his kind guidance, and to Dr. IMAI for his courtesy rendered to the writer during his stay at Onagawa.

DESCRIPTION OF THE SPECIES

Family Homocoelidae DENDY

Genus *Leucosolenia* BOWERBANK1. *Leucosolenia canariensis* (MICHLUCHO-MACLAY)

(Pl. XVII, fig. 1)

Nardoa canariensis, MICHLUCHO-MACLAY, 1868, p. 230.

Nardoa sulphurea, MICHLUCHO-MACLAY, 1868, p. 230.

Nardoa rubra, MICHLUCHO-MACLAY, 1868, p. 230.

Torroma canariensis, HAECKEL, 1870, p. 244.

Torroma rubrum, HAECKEL, 1870, p. 245.

Ascaltis canariensis, HAECKEL, 1872, p. 52, Pl. 9, figs. 1-3; Pl. 10, figs. 1, a-c.

Ascaltis compacta, SCHUFFNER, 1877, p. 404, Pl. 25, fig. 9.

Leucosolenia nansenii, BREITFUSS, 1896, p. 427; 1898, p. 106, Pl. 12, figs. 1-9.

Leucosolenia canariensis, LAKSCHEWITZ, 1886, p. 300, Pl. 7, fig. 1; THACKER, 1908, p. 762, Pl. 40, fig. 3, text-figs. 157-160; DENDY and ROW, 1913, p. 724; HOZAWA, 1918, p. 528; 1933, p. 2, Pl. 1, fig. 1; 1940, p. 134, Pl. 6, fig. 2, text-fig. 2.

Of this well-known species there exists a single specimen (Pl. XVII, fig. 1) in the collection which was collected by Professor HOZAWA from the shore at Rikuzen Enoshima, near Onagawa. The sponge forms an elongated rectangular massive colony, composed of fine net-work of branching and anastomosing Ascon-tubes. The colony is flattened and many small papillae formed of the Ascon-tubes are seen scattered over the surface. The specimen measures about 60 mm. in height, 25 mm. in the greatest breadth, and about 12 mm. in the greatest thickness. The colour is brownish white and the texture is soft.

Localities; — Canary Islands (MICHLUCHO-MACLAY); Cape Verde Islands (THACKER); Mauritius (SCHUFFNER); Minorca (LAKSCHEWITZ); Spitzbergen, Arctic Ocean (BREITFUSS); off Copper Island, Commander Island, off Yuriage, Rikuzen, Mexico (HOZAWA); Enoshima, near Onagawa.

2. *Leucosolenia laxa* KIRK

Leucosolenia laxa, KIRK, 1895, p. 208, Pl. 4, fig. 1; DENDY and ROW, 1913, p. 722; HOZAWA, 1928, pp. 220-221, Pl. 1, figs. 4, 5; 1940, p. 35; TANITA, 1941, p. 2, Pl. I, fig. 1.

This species is represented in the collection by several specimens obtained by a diver from a depth of 15 meters near Hutamatajima, and from a depth of 10 meters off Izushima.

The largest specimen is oval in form, consisting of a massive assemblage of reticulating Ascon-tubes. The total length of the sponge is 20 mm., the greatest breadth about 11 mm., and the thickness 7 mm. in the thickest part. The colour in alcohol is nearly white.

All of the remaining specimens are smaller in size than the first specimen above mentioned and they vary in form, but are nearly the same in general structure.

As these specimens are identical to the type with regard to the inner structure and the spiculation, there is no need to add further descriptions.

Localities: New Zealand (KIRK); Mutsu Bay (HOZAWA, TANITA); Ohshima in Kesennuma Bay, Wajima of Ishikawa Prefecture (HOZAWA); near Hutamatajima in Onagawa Bay, off Izushima near Onagawa.

3. *Leucosolenia mollis*. n. sp.

(Pl. XVII, fig. 2; text-fig. 1)

This new species is based upon seven specimens in the collection. They were obtained by the writer from a depth of about 15 meters in the neighbourhood of Hutamatajima in Onagawa Bay. They are all of a closely similar appearance. Each of them represents an irregularly shaped colony composed of Ascon-tubes and is attached to the substratum. The colony is flattened and the surface shows a loose net-work of Ascon-tubes. The height of the largest specimen (Pl. XVII, fig. 2) which was taken as the type is about 12 mm. and the greatest breadth about 15 mm. The Ascon-tubes are cylindrical and thin-walled, their diameters varying from 0.3 mm. to 2 mm. The osculum is found as a small round aperture at the extremity of each tube. The outer surface of the tubes is minutely hispid.

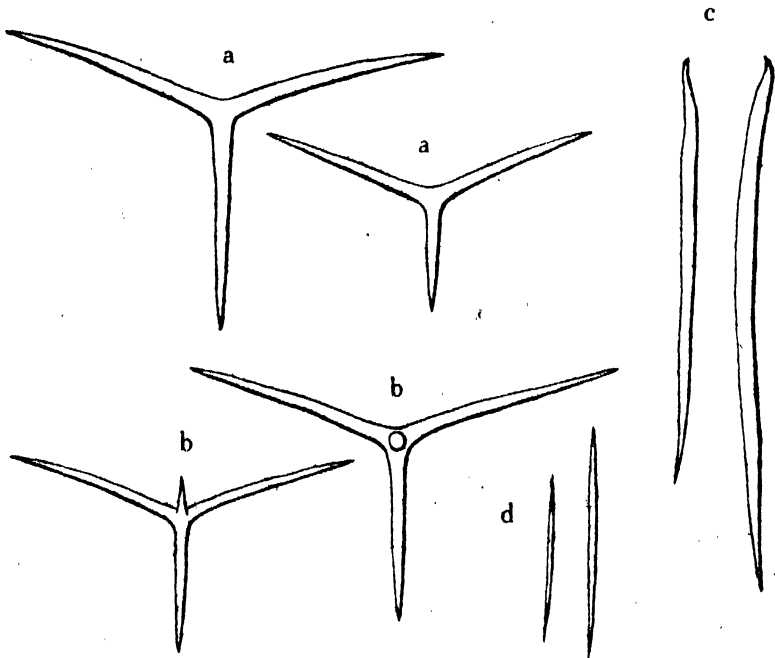
The colour in alcohol is greyish-white, and the texture is very soft and fragile.

Structure; -- The canal system of this species seems to agree with the type *A* proposed by DENDY¹⁾.

¹⁾ DENDY, A. Trans. Roy. Soc. Victoria, Vol. III, 1891, p. 26.

The skeleton is composed of triradiates, quadriradiates, and of oxea. The triradiates and quadriradiates are arranged in several layers lying in the walls of the Ascon-tubes, the apical rays of the latter kind of spicules projecting into the gastral cavity. The oxea are of two kinds, large and small. Large oxea project from the surface of the Ascon-tubes vertically or obliquely. Microxea are more numerous than the large oxea and are densely packed in the mesoderm, their outer ends projecting from the surface.

Spicules (Text-fig. 1):—Triradiates (a) slightly sagittal. All rays slender and equal in thickness. Basal ray straight, tapering to sharp point, either equal to or slightly shorter than paired rays, $70\text{--}130\ \mu$ long and $6\text{--}8\ \mu$ thick at base. Paired rays equal, slightly curved forwards, widely divergent, $90\text{--}140\ \mu$ long and $6\text{--}8\ \mu$ thick at base.



Text-fig. 1. *Leucosolenia mollis*, n. sp. a, triradiates of ascon-tube; b, quadriradiates of ascon-tube; c, large oxea; d, microxea. (a-c $\times 250$; d $\times 400$)

Quadriradiates (b) similar to the triradiates above mentioned, but with a short apical ray. Apical ray curved oralwards, sharply pointed, shorter and slightly thinner than facial rays, $35\text{--}55\ \mu$ long and about $6\ \mu$ thick at base.

Oxea (c) spindle-shaped, slightly curved, broadest in the middle and

tapering towards both ends. The distal end is sharply pointed while the proximal is abruptly curved. They measure 230–400 μ in length and are 7–10 μ thick in the middle parts.

Micoxea (d) straight, sharply pointed at both ends, 35–80 μ long and about 2 μ thick in the middle.

Remarks:—This new species closely resembles *Leucosolenia pilosella* BRØNDSTED¹⁾ and *L. incerta* URBAN²⁾, but it may be easily distinguished from these species by the spiculations.

Locality: Near Hutamatajima in Onagawa Bay.

4. *Leucosolenia mutsu* HOZAWA

Leucosolenia mutsu, HOZAWA, 1928, pp. 219–220, Pl. 1, figs. 1, 2; 1940, p. 35; TANITA, 1940, p. 165, Pl. 8, fig. 1.

Several specimens of this species are contained in the collection obtained from the littoral zone of the two localities of Takenoura in Onagawa Bay and Izushima. Each of them consists of a loose net-work of Ascon-tubes, forming a small irregular mass. The colour of the specimens from Takenoura are greyish-white owing to contamination from mud, while that of the specimens from Izushima are nearly white.

Localities:—Mutsu Bay, Ohshima in Kesennuma Bay (HOZAWA); Matsushima Bay (TANITA); Takenoura in Onagawa Bay, Izushima near Onagawa.

5. *Leucosolenia tenera* TANITA

Leucosolenia tenera, TANITA, 1940, pp. 166–168, Pl. 8, fig. 2, text-fig. 1; 1941, p. 2, Pl. I, fig. 2.

Numerous specimens of this species exist in the collection. They were all secured by the writer from the oyster-beds situated close to the Onagawa Oceano-Chemical Institute, and from a depth of about 15 meters off Izushima.

They are all a closely similar appearance. The sponge forms a loosely branched mass of Ascon-tubes, attached to oyster-shells or other objects.

In general appearance and anatomical structure, the present specimens have the same features as seen in the type which was obtained from Matsushima Bay.

Localities:—Matsushima Bay, Mutsu Bay (TANITA); Onagawa Bay, Izushima.

¹⁾ *Leucosolenia pilosella*, BRØNDSTED, 1928, p. 17, fig. 18.

²⁾ *Leucosolenia incerta*, URBAN, 1908, p. 247.

Family Sycettidae DENDY

Genus Sycon RISSO

6. *Sycon ciliatum* (FABRICIUS)

(Pl. XVII. fig. 3)

Spongia ciliata, FABRICIUS, 1780, p. 448.*Grantia ciliata*, JOHNSTON, 1842, p. 176, Pl. 20, figs. 4, 5; Pl. 21, figs. 6, 7; GRAY, 1867, p. 554.*Sycon giganteum*, HAECKEL, 1870.*Sycocystis oviiformis*, HAECKEL, 1870.*Sycodendrum ramosum*, HAECKEL, 1870.*Sycon ciliatum*, SCHMIDT, 1870, p. 74; BREITFUSS, 1897, p. 216; 1898, p. 18. Pl. 1, figs. 9-12; p. 23; 1927, p. 29; 1932, p. 244; 1936, p. 6; DENDY and ROW, 1913, p. 745; ROW and HOZAWA, 1931, p. 756; BURTON, 1933, p. 236.*Sycandra ciliata*, HAECKEL, 1872, p. 296, Pl. 51, figs. 1 a-t, Pl. 58, fig. 9; ARNESEN, 1901, p. 16.

A single specimen (Pl. XVII, fig. 3) in the collection has been assigned to this species. The sponge forms a solitary tubular individual with a length of 11.5 mm. and the greatest diameter of about 3 mm. The osculum at the upper end of the sponge is surrounded by a well-developed collar 1.5 mm. high. The dermal surface is highly hispid due to the projecting oxea while that of the gastral is slightly rough on account of apical rays of gastral quadriradiates. The colour in alcohol is nearly white.

Localities: — Arctic Ocean, North Atlantic coast of Europe, North America, Adriatic Sea, South-west Australia; Izushima near Onagawa.

7. *Sycon lendenfeldi* ROW and HOZAWA*Sycon lendenfeldi*, ROW and HOZAWA, 1931, pp. 757-768, Pl. 20, fig. 9, text-fig. 8.

This species is represented in the collection by numerous specimens obtained from three different localities. The first group of specimens was collected by the writer from Takenoura and the others were obtained by a diver from a depth of about 15 meters near Hutamatajima and off Izushima. Each of these specimens shows a small solitary individual attached to the substratum by means of a slender stalk. The largest specimen is about 12 mm. high and 2.5 mm. broad. The osculum at the upper end is nearly circular in shape with a diameter of about 1 mm. and surrounded by a fringe 1 mm. high.

Localities: — Fremental District, Albany District of Australia (ROW and HOZAWA); Takenoura and Hutamatajima in Onagawa Bay; off Izushima.

8. *Sycon mundulum* LAMBE

(Pl. XVII, fig. 4)

Sycon mundulum, LAMBE, 1900, pp. 28-29, Pl. 3, fig. 7; DENDY and ROW, 1913, p. 747.

This species is represented in the collection by a single specimen (Onagawa Sp. No. 521) which was taken by Dr. IMAI by means of a dredge from a depth of 8 meters off Miyagasaki in Onagawa Bay.

The sponge (Pl. XVII, fig. 4) forms a tubular solitary individual attached to some foreign object by its base. The length of the specimen is 16 mm. and the greatest breadth about 2 mm. The osculum at the upper end of the body is nearly circular in shape and has a very feebly developed collar. The colour in alcohol is greyish-white.

The canal system and the skeletal structure are exactly similar to those of the type specimen, which was first described by LAMBE. The dimensions of most of the spicules agree with those given in LAMBE's description, though the length of the apical ray of gastral quadriradiates seems to be slightly shorter in the present case than in the type. But as the general appearance, skeleton, spiculation, and other details agree well with the original description, the writer is inclined to identify the specimen from Onagawa Bay with this species.

Localities: --- Davis Strait, Exeter Harbour, off Cape Raper (LAMBE); Onagawa Bay.

9. *Sycon okadai* HOZAWA

Sycon okadai, HOZAWA, 1929, pp. 302-304, Pl. 3, figs. 18, 19, text-fig. 10; TANITA, 1940, p. 168, Pl. 8, fig. 3.

A great number of very perfect specimens of this species are included in the collection. Some of them were obtained by Dr. URAGAMI from Mangoku-ura, near Onagawa, in 1935, and some others were secured by the writer in 1940 from the same locality and also from the oyster-beds of the Onagawa Oceano-Chemical Institute. They are nearly alike in appearance and structure, differing only in size. They vary from 5 mm. to about 50 mm. in length.

Localities: --- Misaki (HOZAWA); Matsushima Bay (TANITA); Mangoku-ura, Onagawa Bay.

10. *Sycon ornatum* KIRK

Sycon ornatum, KIRK, 1897, p. 314, Pl. 31, fig. 2, Pl. 32, fig. 2; DENDY and ROW, 1913, p. 747; BRØNSTED, 1926, p. 303; HOZAWA, 1940, p. 36.

Nine specimens of this species are included in the collection. They are all of a nearly similar appearance. The sponge is attached to the substratum by its base, and shows at the upper end an osculum which is surrounded by a well-developed collar. The collar of the osculum is formed mainly of a very thin membrane in tubular form of nearly equal diameter, but with the uppermost part only diverging slightly towards the outside. The total length of the largest specimen is 9 mm. and the greatest breadth 3 mm. The smallest one measures 4.5 mm. in length.

This species was originally described from a specimen obtained from New Zealand by KIRK in 1897, and after that it was reported by HOZAWA in 1940 as being among the Japanese specimens.

Localities: — New Zealand (KIRK); Rikuzen Ohshima (HOZAWA); off Hutamatajima in Onagawa Bay.

11. *Sycon rotundum*, n. sp.

(Pl. XVII, fig. 5; text-fig. 2)

The collection contains five specimens of this new species. They were collected by the writer in the littoral zone of Takenoura in Onagawa Bay in July, 1939. They vary from 2.5 mm. to 5 mm. in length and from 1.7 mm. to 2.3 mm. in breadth. Each of them represents a solitary individual of a nearly spherical form, showing at the upper end an osculum surrounded by a well-developed collar. It is attached by its base to the shell of the Balanus.

The largest specimen (Pl. XVII, fig. 5) which is taken as the type measures 5 mm. in length and 2.3 mm. in the greatest breadth. The oscular collar is about 2 mm. high. The dermal surface of the sponge is hispid due to the projecting oxea. The gastral surface appears slightly hispid when observed with the hand lens. The sponge is covered with mud, and the colour is yellowish-grey in alcohol.

Structure: — The canal system typical. Flagellate chambers are cylindrical in form, variable in length, straight, not branched, terminating in low rounded distal cones.

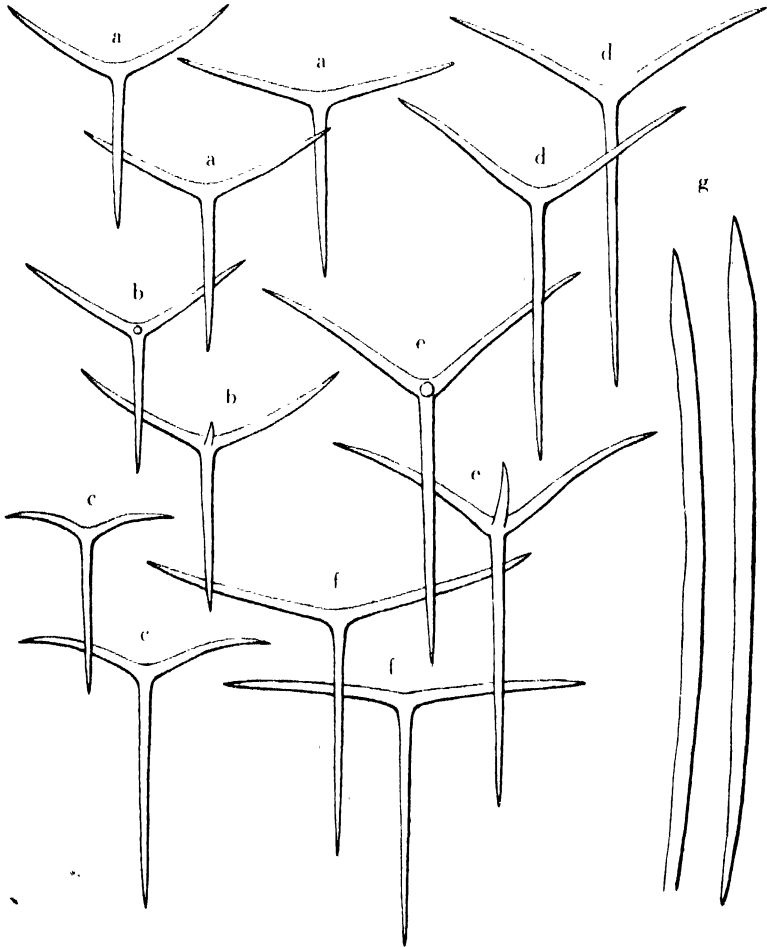
The tubar skeleton is composed of the basal rays of subgastral triradiates and of tubar tri- and quadriradiates which articulate in several layers. The apical rays of the tubar quadriradiates project into the flagellate chamber and are also directed slightly towards the exhalant aperture of the chamber. At the distal ends of the chambers there are set several oxea and hair-like spicules which render a hispid appearance

to the sponge.

The gastral skeleton is made up of paired rays of subgastral triradiates and of gastral triradiates and quadriradiates which are tangentially placed in several layers with their basal rays pointing towards the sponge base.

The oscular margin is consisted of linear spicules and triradiates, all closely set. The former are placed longitudinally forming a thin fringe.

Spicules (Text-fig. 2): -- Tubar triradiates (a) sagittal, variable in shape and size. These spicules situated in the proximal parts of the sponge



Text-fig. 2. *Sycon rotundum*, n. sp. a, tubar triradiates; b, tubar quadriradiates; c, subgastral triradiates; d, gastral triradiates; e, gastral quadriradiates; f, triradiates of oscular margin; g, oxea. (all $\times 210$)

wall are larger than those in the distal and have more widely divergent paired rays. Basal ray straight, sharply pointed, slightly longer than paired rays, $90\text{--}130\ \mu$ long and about $6\ \mu$ thick at base. Paired rays nearly equal, slightly curved, $70\text{--}110\ \mu$ long and $6\ \mu$ thick at base.

Tubar quadriradiates (b) exactly similar to the spicules above mentioned differing only in the presence of an apical ray. Apical ray shorter and slightly thinner than facial rays, $10\text{--}25\ \mu$ long and about $4\ \mu$ thick at base.

Subgastral triradiates (c) sagittal. Basal ray straight, tapering to sharp end, longer than paired rays, $100\text{--}150\ \mu$ long and $6\ \mu$ thick at base. Paired rays equal, widely divergent, $60\text{--}90\ \mu$ long and about $6\ \mu$ thick at base.

Gastral triradiates (d) strongly sagittal, slender-rayed. Basal ray straight, longer than paired rays, $170\text{--}210\ \mu$ long and $6\ \mu$ thick at base. Paired rays slightly curved backwards, $110\text{--}130\ \mu$ long and $6\ \mu$ thick at base.

Gastral quadriradiates (e) have facial rays exactly similar to the gastral triradiates. Apical rays curved upwards, nearly as thick as the facial rays but shorter, $45\text{--}75\ \mu$ long and about $6\ \mu$ thick at base.

Triradiates of oscular margin (f) strongly sagittal. Basal ray straight, sharply pointed, longer and slightly thinner than paired rays, $180\text{--}200\ \mu$ long and $4\text{--}5\ \mu$ thick at base. Paired rays equal, strongly divergent, standing nearly at right angles to the basal ray, $130\text{--}150\ \mu$ long and $6\text{--}7\ \mu$ thick at base.

Oxea at the distal end of flagellate chambers (g) usually slightly curved, tapering towards the both sharply pointed ends, $450\ \mu\text{--}1.2\ \text{mm}$. long and $8\text{--}20\ \mu$ thick in the thickest parts.

Hair-like oxea very fine, more or less curved, attaining a length of over $1.5\ \text{mm}$. by $2\ \mu$ thick.

Linear spicules of oscular collar straight, nearly equally thick in their greater length, sharply pointed at both ends, about $2\ \text{mm}$. long and $2\text{--}5\ \mu$ thick in the middle parts.

Remarks: — *Sycon munitum* JENKIN¹⁾ and *S. maximum* (HAECKEL)²⁾ seem to be closely allied to this new species. But from this new species the former species is differentiated in having two sorts of gastral quadriradiates, while the latter may be easily distinguished by the difference in the shapes and dimensions of the spicules.

As only a few species of *Sycon* are known to be provided with quadri-

¹⁾ *Sycon munitum*, JENKIN, 1908, p. 443, fig. 91.

²⁾ *Sycon arctica* var. *maxima*, HAECKEL, 1872, p. 354, Pl. 55, fig. 1; Pl. 60, fig. 15.

radiates in the articulated tubar skeleton, it is very easy to distinguish the present species from the other members of the same genus.

Locality: — Takenoura in Onagawa Bay.

12. *Sycon simushirensis* HOZAWA

Sycon simushirensis, HOZAWA, 1918, pp. 529-531, Pl. 84, fig. 6, text-fig. 2.

The collection contains four specimens of this species. Each of them represents a small solitary tubular individual, provided with a nearly circular osculum at the upper end. The largest specimen is 8 mm. high and about 2 mm. broad. The dermal surface is very slightly hispid, while the gastral appears nearly smooth to the naked eye. The colour in alcohol is greyish-white.

Localities: — Simushir Island (HOZAWA); near Hutamatajima in Onagawa Bay.

Family *Heteropiidae* DENDY

Genus *Vosmaeropsis* DENDY

13. *Vosmaeropsis maculata* HOZAWA

(Pl. XVII, fig. 6)

Vosmaeropsis maculata, HOZAWA, 1929, pp. 321-324, Pl. 5, figs. 32, 33, text-fig. 17.

There are two specimens of this species in the collection which were taken near Hutamatajima in Onagawa Bay. Each of them represents a solitary and strongly laterally compressed individual, provided with a naked slitlike osculum at the upper end. The largest specimens (Pl. XVII, fig. 6) is about 10 mm. long and 3 mm. broad. The dermal surface appears smooth, and the colour is nearly white.

In general appearance and anatomical structure, the present specimens are identical with the type.

Localities: — Misaki, Enoura in Suruga Bay (HOZAWA); near Hutamatajima in Onagawa Bay.

Family *Grantiidae* DENDY

Genus *Leucandra* HAECKEL

14. *Leucandra abratsbo* HOZAWA

(Pl. XVII, fig. 7)

Leucandra abratsbo, HOZAWA, 1929, pp. 359-362, Pl. 9, figs. 57, 58, text-fig. 29; 1940, p. 53.

The collection contains numerous specimens of this species, of which four (Onagawa Sp. No. 482 & 484) were obtained by Dr. IMAI at Rikuzen Enoshima, six by Professor HOZAWA from the same locality, and the remaining ones by the writer at Hutamatajima. They vary in size and shape. The largest one (Pl. XVII, fig. 7) measures 25 mm. in length and 10 mm. in breadth. It has two circular oscula at the upper end, while the remaining specimens have only one osculum each. The colour of the specimens varies from greyish-white to nearly white.

Localities: — Misaki, Wajima (HOZAWA); Enoshima and Hutamatajima in Onagawa Bay.

15. *Leucandra mediocanellata* HOZAWA

(Pl. XVII, fig. 8)

Leucandra mediocanellata, HOZAWA, 1940. pp. 53-56, Pl. 4, fig. 7, text-fig. 9.

The collection contains three specimens of this species, which were obtained by Professor HOZAWA near the shore of Rikuzen Enoshima in 1940. Each of them represents a small solitary individual of nearly spherical shape with a well-developed oscular collar at its upper end.

The largest specimen (Pl. XVII, fig. 8) is 13.5 mm. in length and about 7 mm. in diameter. The dermal surface is highly hispid due to the projecting oxea and the gastral surface slightly hispid from the apical rays of gastral quadriradiates. The colour in alcohol is greyish-white and the texture slightly rigid.

Localities: — Rikuzen Ohshima (HOZAWA); Rikuzen Enoshima.

16. *Leucandra tomentosa* TANITA

Leucandra tomentosa, TANITA, 1940, pp. 174-176, Pl. 8, fig. 6, text-fig. 4; 1941, p. 3, Pl. I, fig. 4.

Numerous specimens obtained from Takenoura, Hutamatajima, and Izushima are referable to this species. They range in height from 5 to 15 mm. As in the case of the type specimen, the outer surface is very strongly hispid due to the projecting long oxea. Each of these specimens has a well-developed collar at the upper end.

Localities: — Matsushima Bay, Mutsu Bay (TANITA); Takenoura and Hutamatajima in Onagawa Bay, off Izushima.

17. *Leucandra valida* LAMBE

(Pl. XVII, fig. 9; text-fig. 3)

Leucandra valida, LAMBE, 1900, pp. 32-33, Pl. 4, fig. 10, Pl. 5, fig. 11; DENDY and ROW, 1913, p. 771.

This species is represented in the collection by numerous specimens obtained from the three localities of Takenoura, Hutamatajima, and Izu-shima. They are all of a closely similar appearance. Each of them represents a solitary individual of a sub-cylindrical or slightly laterally compressed tubular form, showing an osculum surrounded by a developed collar at the upper end.

The largest specimen (Pl. XVII, fig. 9) is about 17 mm. long and 6.5 mm. broad near the base. The dermal surface of the sponge is highly hispid owing to the oxea projecting from it but the gastral appears nearly smooth to the naked eye. The sponge-wall is about 2.3 mm. in the thickest parts of the body. The osculum is surrounded by a collar of about 1 mm. diameter and 2.7 mm. high. The colour in alcohol is greyish-white and the texture is elastic.

Structure: — The canal system is leuconoid. The flagellate chambers, subspherical or ovoid in shape, are set together in the chamber layer. The dermal skeleton is made up of triradiates placed tangentially in several layers. Oxea placed at nearly right angles to the dermal surface, have their proximal parts embedded deeply in the body-wall and their distal parts projecting far beyond the surface. Moreover, hair-like spicules are found on the surface placed in a similar manner as the oxea.

The skeleton of chamber layer consists of the basal rays of subgastral triradiates and of tubar triradiates, which are variable in size and are irregularly set together. The gastral skeleton is composed of paired rays of subgastral triradiates and of gastral tri- and quadriradiates.

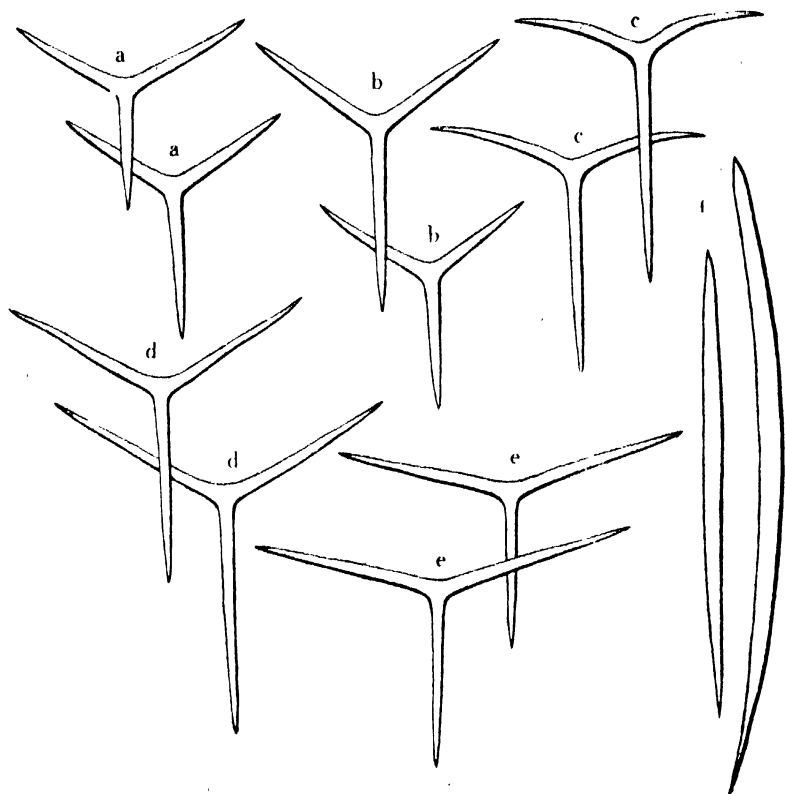
The oscular margin is consisted of tangentially placed triradiates and of linear spicules forming a distinct collar.

Spicules (Text-fig. 3): — Dermal triradiates (a) slightly sagittal. All rays are of nearly equal thickness lying in the same plane. Basal ray straight, sharply pointed, either nearly equal to or slightly longer than paired rays, 140-240 μ long and 12-18 μ thick at base. Paired rays equal, straight or slightly curved, 140-220 μ long and 12-18 μ thick at base.

Tubar triradiates (b) slightly sagittal and variable in size, but are nearly same as the dermal triradiates in shape. Basal ray straight, slightly longer than paired rays, 160-250 μ long and 12-16 μ thick at base. Paired

rays nearly equal, 150–230 μ long and 12–16 μ thick at base.

Subgastral triradiates (c) strongly sagittal. Basal ray straight, sharply pointed, longer than paired rays, 250–280 μ long and 12–16 μ thick at base. Paired rays curved backwards, widely divergent, 110–220 μ long and 12–16 μ thick at base.



Text-fig 3. *Leucandra valida* LAMBE. a, dermal triradiates; b, tubar triradiates; c, subgastral triradiates; d, gastral triradiates; e, triradiates of oscular margin; f, oxea. (a–e $\times 120$; f $\times 60$).

Gastral triradiates (d) sagittal. Basal ray straight, longer than paired rays, 270–380 μ long and 8–12 μ thick at base. Paired rays nearly equal, slightly curved, 180–270 μ long and 8–12 μ thick at base.

Gastral quadriradiates nearly same in size and shape as the gastral triradiates, but differ in the presence of apical rays. Apical ray nearly straight, sharply pointed, shorter and thinner than facial rays, 50–100 μ long and 8–10 μ thick at base.

Triradiates of the oscular margin (c) strongly sagittal. Basal ray straight, shorter and thinner than paired rays, 180-240 μ long and about 8 μ thick at base. Paired rays equal, widely divergent, nearly making right angles with the basal ray, 270-320 μ long and 10-12 μ thick at base.

Large oxea (f) elongate spindle-shaped, more or less curved, sharply pointed at both ends, but the distal parts are usually stouter than the proximal, 820 μ -1.8 mm. long and 25-60 μ thick at the thickest parts.

Hair-like dermal oxea are very slender, straight or nearly so, measuring up to 550 μ in length and about 4 μ in thickness.

Linear spicules of the oscular margin straight, nearly uniformly thick throughout their entire length, attaining a length of 2.5 mm. and the thickness of 2-6 μ .

Remarks: --- Comparing the present specimens with the type of this species, the dimensions of gastral radiates shown by the present specimen appear to be slightly thinner than those by the type, and moreover the shape of subgastral triradiates seems to differ slightly. But in other details the present specimens seem to agree well with the original description of this species.

Localities: --- Davis Strait, Exeter Harbour (LAMBE); Takenoura and Hutamatajima in Onagawa Bay, off Izushima.

18. *Leucandra vermiformis*, n. sp.

(Pl. XVII, fig. 10; text-fig. 4)

Only a single specimen of this new species exists in the collection. It was collected from a depth of about 15 meters near Hutamatajima in Onagawa Bay. It is a solitary tubular individual and is irregularly bent in worm-like manner.

It measures about 13 mm. in total length and 7 mm. in the greatest breadth, the wall reaching about 1 mm. in thickness. The osculum at the upper end is nearly naked and circular in shape with a diameter of 1.5 mm. The dermal surface is fairly hispid due to the projecting oxea, while the gastral seems nearly smooth to the naked eye.

The colour in alcohol is white, and the texture is moderately firm.

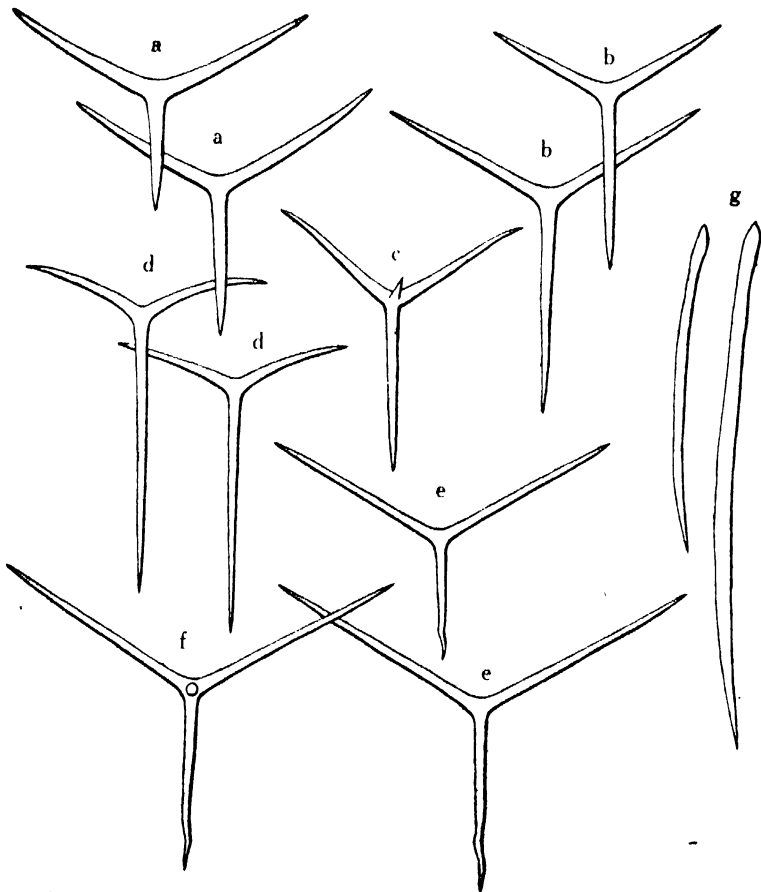
Structure: --- The canal system is of the leuconoid type. The dermal skeleton is composed of triradiates which are placed tangentially with the basal ray pointing downwards. A number of large oxea and hair-like spicules are placed nearly perpendicularly to the dermal surface. They project to some extent distally beyond the surface and their proximal

parts are embedded in the chamber layer.

Tubar skeleton is made up of tubar triradiates, basal rays of subgastral triradiates, and proximal parts of large oxea. The walls of the larger exhalant canals are lined with several quadriradiates with their apical rays projecting into the canal.

The gastral skeleton is thinner than the dermal and is consisted of gastral tri- and quadriradiates placed tangentially in a thin layer and of paired rays of subgastral triradiates.

Spicules (Text-fig. 4): — Dermal triradiates (a) slightly sagittal. All rays are nearly equally thick and sharply pointed. Basal ray straight,



Text-fig. 4. *Leucandra vermiformis*, n. sp. a, dermal triradiates; b, tubar triradiates; c, quadriradiates of larger exhalant canal; d, subgastral triradiates; e, gastral triradiates; f, gastral quadriradiates; g, oxea. (a-f $\times 120$; g $\times 60$).

usually shorter than paired rays, 120–190 μ long and 12–18 μ thick at base. Paired rays nearly equal, slightly curved forwards, 150–230 μ long and 12–18 μ thick at base.

Tubar triradiates (b) sagittal. Basal ray straight, gradually and sharply pointed, longer than paired rays, 210–260 μ long and 12–16 μ thick at base. Paired rays equal, nearly straight, 180–220 μ long and 12–16 μ thick at base.

Quadriradiates of the larger exhalant canals (c) sagittal. Basal ray straight, 180–240 μ long and 10–16 μ thick at base. Paired rays slightly curved backwards, nearly equal, 130–170 μ long and 10–16 μ thick at base. Apical ray short, sharply pointed, 50–75 μ long and about 12 μ thick at base.

Subgastral triradiates (d) strongly sagittal. Basal ray straight, tapering to sharp point, longer than paired rays, 200–300 μ long and 10–14 μ thick at base. Paired rays equal, widely divergent, curved backwards, 140–175 μ long and 10–14 μ thick at base.

Gastral triradiates (e) sagittal, rather slender rayed. Basal ray shorter than paired rays, irregularly curved near the sharp end, 170–210 μ long and 8–10 μ thick at base. Paired rays nearly straight, 220–260 μ long and 8–10 μ thick at base.

Gastral quadriradiates (f) exactly similar to the gastral triradiates, differing only in the presence of the apical ray. Apical ray slightly curved upwards, shorter than facial rays, about 70 μ long and 8 μ thick at base.

Large oxea (g) slender, slightly curved, sharply pointed at both ends, but the distal end slightly lance-headed. They measure 750 μ –2 mm. in length and 25–50 μ in the greatest breadth.

Hair-like spicules slender, nearly uniformly thick in the greater parts of their length, generally with the free end broken off. An example of the spicules measured 1.5 mm. long and 3 μ thick.

Remarks: — The present species closely resembles *Leucandra thulakomorpha* Row and HOZAWA¹⁾, but it may be easily distinguished from it by the absence of dermal quadriradiates.

Locality: — near Hutamatajima in Onagawa Bay.

LITERATURE CITED

- ARNESSEN, E. (1901). *Spongeir fra norske Kyst. I. Calcarea. Systematisk Katalog med bemerkninger og-bestemmelsestabel.* Bergens Mus. Aarbog, 1900, No. 5.
- BREITFUSS, L. (1896). *Kalkschwämme der Bremer-Expedition nach Ost-Spitzbergen im Jahre 1894.* Zool. Jahrb., 13, 1–100.

¹⁾ *Leucandra thulakomorpha*, Row and HOZAWA, 1931, p. 791, Pl. 21, fig. 15, text-fig. 14.

1889. Zool. Anz., Bd. XIX, No. 514, pp. 426-432.
- (1897). Katalog des Calcareea der zoologischen Sammlung des Königlichen Museums für Naturkunde zu Berlin. Arch. f. Naturgesch., Jahrgang LXIII, Bd. 1, pp. 205-226.
- (1898). Die Kalkschwammfauna von Spitzbergen. Nach den Sammlungen der Bremer Expedition nach Ost-Spitzbergen im Jahre 1889 (Prof. W. KÜKENTHAL und Dr. A. WALTER). Zoolog. Jahrbuch. syst. Abth., Bd. XI, pp. 103-120, Taf. 12, 13.
- (1927). Die Kalkschwammfauna der Nord- und Ostsee. Zool. Anz., Bd. LXX. pp. 26-36.
- (1932). Die Kalkschwammfauna des arktischen Gebietes. Fauna arctica, Bd. VI, No. 3, pp. 235-252.
- (1936). Kalkschwämme vom Skagerrak und Kaltegat unter Berücksichtigung ihrer Weltverbreitung. Göteborgs Kungl. Vetenskaps Vitt. Sam. Handl., Ser. B. Bd. 4, No. 15, pp. 1-16.
- BRØNDSTEDT, H. V. (1926). Papers from Dr. Th. MORTENSEN's Pacific Expedition 1914-16. XXXV. Sponges from New Zealand. Part II. Saertryk af Vidensk. Medd. fra Dansk Naturh. Foren. Bd. 81, pp. 295-331.
- (1928). Die Kalkschwämme der deutschen Südpolar Expedition 1901-1903. "Deutsche Südpolar-Expedition" XX, pp. 1-47.
- BURTON, M. (1933). Report on a small Collection of Sponges from Stil Bay. S. Africa. Ann. Mag. Nat. Hist., Ser. 10, Vol. XI, pp. 235-244.
- DENDY, A. (1891). Monograph of Victorian Sponges. Part 1. The Organization and Classification of the Calcareea Homocoela, with Descriptions of the Victorian Species. Trans. Roy. Soc. Vict., Vol. III, No. 1, pp. 1-82, Pls. 1-9.
- DENDY, A. and ROW, W. H. (1913). The Classification and Phylogeny of the Calcareous Sponges, with a Reference List of all the described Species, systematically arranged. Proc. Zool. Soc. London, 1913, pp. 704-813.
- FABRICIUS, O. (1780). Fauna Groenlandica. Hafniae at Lipsiae, 1780.
- GRAY, J. E. (1867). Notes on the arrangement of Sponges, with the Description of some new genera. Proc. Zool. Soc. London, 1867, pp. 492-558, Pls. XXVII-XXVIII.
- HAECKEL, E. (1870). Prodromus eines Systems der Kalkschwämme. Jenaische Zeitsch., Bd. V, pp. 236-254.
- (1872). Die Kalkschwämme, eine Monographie. Berlin.
- HOZAWA, S. (1918). Report on the Calcareous Sponges collected by the U. S. Fisheries Steamer "Albatross" in the North-western Pacific during 1906. Proc. U. S. Nat. Mus., Vol. 54, pp. 525-556, Pls. 84, 85.
- (1928). Report of the Biological Survey of Mutsu Bay. 6. Calcareea of Mutsu Bay. Sci. Rep. Tōhoku Imp. Univ., Biol. Vol. III, No. 2, pp. 219-222, Pl. I.
- (1929). Studies on the Calcareous Sponges of Japan. Journ. Fac. Sci. Imp. Univ. of Tokyo, Sec. IV, Zool., Vol. I, Part 5, pp. 277-389, Pls. I-XII.
- (1933). Report on the Calcareous Sponges obtained by the Survey of the Continental Shelf bordering of Japan. Sci. Rep. Tōhoku Imp. Univ., Biol. Vol. VIII, No. 1, pp. 1-20, Pl. I.
- (1940). On some Japanese Calcareous Sponges. Sci. Rep. Tōhoku Imp. Univ., Biol. Vol. XV, No. 1, pp. 29-58, Pls. IV, V.
- (1940). Report on the Calcareous Sponges obtained by the Zoological Institute and Museum of Hamburg. Sci. Rep. Tōhoku Imp. Univ., Biol. Vol. XV, No. 2, pp. 131-163, Pls. VI, VII.

- JENKIN, C. F. (1908). The Marine Fauna of Zanzibar and British East Africa, from Collections made by CYRIL CROSSLAND, M. A., in the years 1901 and 1902. The Calcareous Sponges. Proc. Zool. Soc. London, 1908, pp. 434-456.
- JOHNSTON, G. (1842). A History of British Sponges and Lithophytes. Edinburgh.
- KIRK, H. B. (1895). New Zealand Sponges: Third Paper. Trans. New Zealand Inst., Vol. XXVIII, pp. 205-210, Pls. III, IV.
- (1897). Notes on New Zealand Sponges: Fourth Paper. Trans. New Zealand Inst., Vol. XXX, pp. 313-316, Pls. XXXI, XXXII.
- LACKSCHWITZ, P. (1886). Über die Kalkschwämme Menorcas. Zool. Jahrbuch., Bd. I. pp. 291-310. Taf. VII.
- LAMBE, L. M. (1900). Sponges from the Coasts of Northeastern Canada and Greenland. Trans. Roy. Soc. Canada, Ser. 2, Vol. VI, Sect. 4, pp. 19-49, Pls. I-VI.
- MICHLUCHO-MACLAY, N. De. (1868). Beiträge zur Kenntniss der Spongien. Jenaische Zeitsch., Bd. IV, pp. 221-240.
- ROW, W. H. and HOZAWA, S. (1931). Report on the Calcareous obtained by the Hamburg South-West Australian Expedition of 1905. Sci. Rep. Tōhoku Imp. Univ., Biol. Vol. VI, No. 4, pp. 727-818, Pls. XIX-XXI.
- SCHMIDT, O. (1870). Grundzüge einer Spongien Fauna des Atlantischen Gebietes. Leipzig, 1870.
- SCHUFFNER, O. (1877). Beschreibung einiger neuer Kalkschwämme. Jenaische Zeitsch., Bd. XI, pp. 403-433, Taf. XXIV-XXVI.
- TANITA, S. (1940). Calcareous Sponges of Matsushima Bay. Sci. Rep. Tōhoku Imp. Univ., Biol. Vol. XV, No. 2, pp. 165-178, Pl. VIII.
- (1941). Report of the Biological Survey of Mutsu Bay. 35. Studies on the Calcareous of Mutsu Bay. Sci. Rep. Tōhoku Imp. Univ., Biol. Vol. XVI, No. 1, pp. 1-8, Pl. 1.
- THACKER, A. G. (1908). On Collection of the Cape Verde Islands Fauna made by CYRIL CROSSLAND, 1904, the Calcareous Sponges. Proc. Zool. Soc. London, 1908, pp. 757-782, Pl. XI.
- URBAN, F. (1908). Die Kalkschwämme der deutschen Tiefsee Expedition. Zool. Anz., Bd. XXXIII, pp. 247-252.
- (1909). Die Calcareous. Wiss. Ergeb. deutsch. Tiefsee-Exp. (Valdevia). Bd. XIX, Jena, 1909.

EXPLANATION OF PLATE XVII

- Fig. 1. *Leucosolenia canariensis* (MICHLUCHO-MACLAY). Natural size.
Fig. 2. *Leucosolenia mollis*, n. sp. about $\times 1.5$.
Fig. 3. *Sycón ciliatum* (FABRICIUS). about. $\times 2$.
Fig. 4. *Sycon mundulum* LAMBE. about. $\times 2$.
Fig. 5. *Sycon rotundum*, n. sp. about. $\times 1.5$.
Fig. 6. *Vosmaeropsis maculata* HOZAWA. about $\times 1.5$.
Fig. 7. *Leucandra abratsbo* HOZAWA. about $\times 2$.
Fig. 8. *Leucandra mediocanellata* HOZAWA. about $\times 2$.
Fig. 9. *Leucandra valida* LAMBE. about $\times 1.5$.
Fig. 10. *Leucandra vermiformis*, n. sp. about $\times 1.5$.



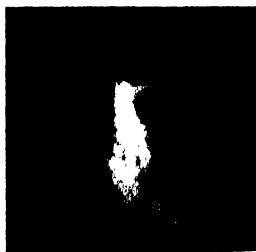
1



2



4



3



5



8



6



7



9



10

TANITA photo.

S. TANITA : Calcareia from Onagawa Bay and its Vicinity.

STUDIES OF CLEAVAGE II. CLEAVAGE OF EGGS OF A SEA URCHIN, *STRONGYLOCENTROTUS PULCHERRIMUS*, IN CALCIUM-FREE SEA WATER¹⁾

By

ISAO MOTOMURA

Biological Institute, Tôhoku Imperial University, Sendai

(With Plate XVIII)

(Received January 20, 1941)

It has been shown previously (MOTOMURA 1935, 1936) that the cell boundary between the blastomeres of the developing eggs of *Strongylocentrotus pulcherrimus* in normal sea water is newly produced in the course of cleavage, and that the new formation does not occur in calcium-free sea water. Those results are based on the observation of the behavior of the cortical cytoplasm, which contains the characteristic pigment granules. Recently, DAN, DAN and YANAGITA (1938) also ascertained the new formation of the cell surface in the furrow region in the eggs of *Astriclypeus* in sea water with their special method of particle-marking. And, on the other hand, the results of the present writer's observations on the cleavage in calcium-free sea water were not accepted by DAN and DAN, who advocate the new formation in this artificial medium, as well as in sea water (1940).

It is the purpose of this account first, to supplement the original, brief description on the cleavage of the egg of *Strongylocentrotus pulcherrimus* in calcium-free sea water given by the present writer in 1935 and, second, to contribute to a better understanding of the remarkable phenomena of the cleavage in this artificial medium. The discussion includes (1) the reliability of the pigment granules as the mark of the cortical cytoplasm, (2) the distinction between the pigment granules and the osmium-blackened granules, (3) the effect of the number of washings in calcium-free sea water, and (4) the presence of a radial halo-layer on the surface of fertilized eggs washed in calcium-free sea water.

MATERIAL AND METHOD

The observations were carried out during March and April, 1940 at

¹⁾ These researches were aided by a grant from Japan Society for the Promotion of Scientific Research.

Misaki and Asamusi Marine Biological Stations. HERBST's artificial calcium-free sea water of the following composition was used; NaCl 26.9 grms, KCl 0.7 gm, $\text{MgSO}_4(7\text{H}_2\text{O})$ 11.9 grms, NaHCO_3 0.45 gm, and distilled water up to 1000 cc. All reagents used were Dog Brand Guaranteed Reagents of KONISHI & Co., Tokyo. 1 cc. of saturated aqueous solution of phenol red was added to every 5 litres of calcium-free sea water as the indicator for controlling the pH value to 8.2. For the observation of the yellow pigment granules in the cortical cytoplasm a hand made blue filter was used. This was a sheet of cellophane slightly stained with 0.1% aqueous solution of Brillantcresylblau (GRÜBLER) and mounted with canada balsam between two glass plates. A tricolor blue filter, WRATTEN No. 49, was also used for photographing. The filters were inserted between the microscopic condenser and the cooling vessel of the light source.

OBSERVATIONS

A. *Behavior of the cortical cytoplasm in the course of cleavage in calcium-free sea water.*

The egg was seminated in a small dish containing 20 cc. of sea water. Three minutes after fertilization the fertilization membrane was removed by shaking the egg in a test tube half full of calcium-free sea water. The fertilization membrane of this species attains its maximum extension three minutes after fertilization at a room temperature of 13°C , and if the egg remained longer in sea water the membrane became too tough to remove without injuring the egg. The eggs were, then, washed 15 times repeatedly in calcium-free sea water until before the beginning of the first cleavage. This procedure is absolutely necessary because calcium-ions contained in the egg cytoplasm come out into the external medium, and they contaminate the calcium-free sea water. When the slight constriction of the first cleavage furrow appeared, the eggs were sealed in a depression slide with a small amount of calcium-free sea water.

The cleavage furrow appeared first 100 minutes after fertilization. As the furrow is going on the blastomeres separated from each other, and were connected with a long protoplasmic bridge, which reached up to 40μ in maximum length. 20 minutes after the beginning of the first cleavage the bridge was broken up into many drops of cytoplasm. The distance between the blastomeres seemed to become longer at this stage, and before the beginning of the second cleavage it became slightly

shorter. 150 minutes after fertilization the furrow of the second cleavage appeared.

As reported in the previous paper (1935) the cortical cytoplasm of the egg of this species contains yellow pigment granules, which appear dark red in blue light filtered with WRATTEN No. 49, and orange yellow with a Brillantcresylblau filter. At the beginning of the cleavage the pigment granules were observed on the whole surface of the egg; that is, not only on the surface of the blastomeres but also in the protoplasmic bridge. And they clouded at the point where the bridge was attached to the blastomeres. This shows that at the beginning of the cleavage the increasing surface is covered by the extension of the cortical cytoplasm of the uncleaved egg. The protoplasmic bridge soon broke down into many small drops, in which the pigment granules were also observable. The cluster of the pigment granules at the side of the bridge attachment of the blastomeres was also observed at this stage, but it soon became indistinct, and the whole surface of the blastomeres was covered with pigmented cortical cytoplasm of a nearly equal thickness (Figs. 1 and 2). In some eggs the blastomeres showed the amoeboid movement of the cytoplasm at the side of the bridge attachment at 30 to 35 minutes after the appearance of the first cleavage furrow (Fig. 3). And after this, the blastomeres again rounded up. In some cases the cluster of the pigmented cortical cytoplasm appeared at the bridge attachment (Fig. 4). But soon the thickness of the cortical cytoplasm became equal on all sides forming a closed ring of the pigmented cortical cytoplasm in an optical section (Fig. 5). When the second cleavage had begun the pigmented cortical cytoplasm was observed at the side of the bridge attachment as well as at the polar side of the first cleavage (Figs. 6, 7 and 8).

The above facts show that the pigmented cortical cytoplasm always covers the whole surface of the blastomeres until the beginning of the second cleavage. Although the cortical cytoplasm varies in thickness in the course of the cleavage, it finally attains an equal thickness just before the second cleavage. Thus, the pulsation of the cortical cytoplasm after the appearance of the first cleavage furrow is a remarkable phenomenon in the cleavage in calcium-free sea water.

B. *Radial halo-layer.*

In calcium-free sea water a radial halo-layer is observable on the surface of the fertilized egg. It is a fine structure of thread-like substance

attaching radially to the surface of the egg (Fig. 12), and it can be ascertained by photographing. Probably it will be only the remaining portion of the hyaline membrane, most of which has been dissolved away with calcium-free sea water. For this reason the radial structure is also observable in the hyaline layer of the fertilized egg in sea water.

The radial halo-layer covered the whole surface of the fertilized un-cleaved egg as well as that of the blastomeres. And at the side of the bridge attachment it was also visible until the beginning of the second cleavage (Figs. 5, 6 and 7). If it is possible to assume that the radial halo-layer is present only on the old surface of the egg, the above fact reveals the extension of the old surface in the course of the first cleavage in calcium-free sea water.

C. Effect of the number of washings with calcium-free sea water.

In the experiment mentioned above the eggs were washed 15 times before the beginning of the first cleavage. To test the effect of washing, portions of the eggs from the same lot were separated into other glass vessels containing calcium-free sea water when the stock lot was washed 3, 6 and 10 times respectively. And four vessels, including the last one, which contained the eggs washed 15 times as described in the above mentioned experiment, remained untouched for eight hours, after which the eggs were photographed. The eggs washed only three times showed the remarkable tendency of the blastomeres to cling together, forming irregular masses of cells (Fig. 9). The eggs washed six times formed loose masses of blastomeres in comparison with the former case (Fig. 10). The eggs washed ten or fifteen times showed an equal tendency not to cling, that is the blastomeres scattered on the bottom of the glass vessel (Fig. 11).

Thus, when the eggs are washed more than ten times in calcium-free sea water at regular intervals before the beginning of the first cleavage, they lose the tendency of clinging, which is noticeable in the presence of the hyaline membrane in sea water. The different effects, depending on the number of washings, were observable even in the early cleavage stages. For instance in the first cleavage, the blastomeres did not form the long protoplasmic bridge, but clung easily to each other after the deep cleavage furrow had been formed. This may have been caused by the presence of calcium-ions, which came probably from the egg cytoplasm. Of course, the purity of the calcium-free sea water is of first importance in this experiment, but the maximum amount of calcium-ions tolerable

for the experiment was not estimated by the method of chemical analysis.

DISCUSSION

1) *Reliability of the pigment granules as the mark of the cortical cytoplasm.*

It was pointed out by the present writer in 1935 that yellow pigment granules in the egg of this species are useful as a mark of the cortical cytoplasm, because they are not displaced by the strong centrifugal force of about 14000 times gravity. And tracing the behavior of the yellow pigment granules in the course of cleavage, the movement of the cortical cytoplasm can be deduced. Recently DAN and DAN (1940) questioned the reliability of the granules as a mark. They stated that, "when two fertilized eggs with perfectly complete rings of dark red granules are brought into contact, the granules appear to be absent from the parts of the egg peripheries which are in contact. Therefore since the visibility of the dark red ring with a filter appears to be largely dependent upon the optical conditions, it must be concluded that one cannot safely determine the presence or absence of granules on the sole basis of observations with filter". But it is difficult for the present writer to understand why the results of his experiment, described in the previous paper (1935 Figs. 72-75), should be nullified by their experiment cited above. Namely, the present writer has already ascertained the absence of the pigment granules on the contact surfaces of the blastomeres by means of the maceration experiment with calcium-free sea water at the two-, four- and eight-cell stage of the normal eggs respectively. And it will be clear that this experiment was designed for the purpose of avoiding the optical illusion, or the disturbance caused by the spatial relations of the blastomeres. That is, the blastomeres closely packed with hyaline membrane were separated from each other by dissolving the hyaline membrane with calcium-free sea water, and they assumed a quite spherical form. Thus the actual distribution of the pigment granules in the egg developed in sea water was determined, avoiding the optical illusion, and therefore the result was quite different from that in which the eggs were developed in calcium-free sea water (1935 pp. 235-239).

On the other hand, in the eggs cultured in and washed repeatedly with calcium-free sea water the pigment granules were observed on the whole surface of the blastomeres, that is, not only on the polar region, but also on the furrow side of the blastomeres. As the blastomeres separated widely from each other in the last case, it is evident, at least,

that the presence of the pigment granules can not be denied on the grounds of optical illusions.

2) *The distinction between the pigment granules and the osmium-blackened granules.*

It was observed by DAN and DAN (1940) that in sections of eggs fixed with osmium mixtures a row of blackened granules extended completely around the blastomeres cleaving in sea water. They assumed the identity of the pigment granules in living eggs and the osmium-blackened granules in sections, and concluded that the osmium-blackened granules, which are identical with the pigment granules according to their opinion, are present all over the surface of the blastomeres at any time of cleavage. This conclusion is also stated in support of the view that the visibility of the pigment granules is dependent upon the optical conditions. But, unfortunately, the writer cannot accept DAN's assumption, because the osmium-blackened granules appear to be a sort of endoplasmic granules, which are easily displaced by centrifuging, while the pigment granules of this species are not detached from the cortical cytoplasm by a centrifugal force of less than 14000 times gravity for three minutes. And, moreover, it is well known by histologists that the results of osmium-staining differ according to the concentration of fixatives and according to the length of time of impregnation. The writer believes that the osmium-blackened granules described by DAN and DAN are not identical with the yellow pigment granules in the living egg.

As to the reliability of the pigment granules as a mark of the cortical cytoplasm, the writer does not find any reason to alter his previous opinion. And the presence of the pigment granules on the whole surface of the blastomeres of the egg developing in calcium-free sea water is an indication of the presence of the cortical cytoplasm covering the whole surface of the blastomeres.

3) *The effect of the number of washings with calcium-free sea water.*

It is obvious that in the experiments with calcium-free sea water great care must be taken not to contaminate the medium by impure calcium-ions. The experiment mentioned above showed that when the eggs were washed many times with calcium-free sea water the blastomeres lost their capacity for clinging together. And when the eggs were washed only a few times the blastomeres retained this capacity. This was caused mainly by the presence of a small amount of calcium-ions, which came from the egg cytoplasm and were detected by adding aqueous solution of ammonium oxalate. It has been shown by HERBST (1900) that in *Echinus micro-*

tubercuratus the blastomeres separated from each other more widely than the radius when the eggs are cultured in calcium-free sea water. The writer also observed the same tendency in the eggs of *Strongylocentrotus pulcherrimus*. And, on the other hand, the figures shown by DAN and DAN (1940, Plate I, Fig. E and Textfig. 2) indicate the tendency of the blastomeres to cling. The last case seems to be identical with the writer's cases of incomplete washing with calcium-free sea water. This assumption may be ascertained from their own words, that is, "in calcium-free sea water alone the blastomeres tend to come into close contact during interkinesis, rendering observation difficult, while this does not occur if excess KCl is present, although in all other respect the cleavage picture is the same".

4) *Presence of the radial halo-layer on the surface of the fertilized egg washed in calcium-free sea water.*

In the fertilized egg of *Echinus microtuberculatus* a radial halo-layer, strahlige Hautschicht, became visible, when the egg was washed with calcium-free sea water (HERBST 1900). According to HERBST this layer is the transformed hyaline membrane caused by the lack of calcium-ions. He found the layer not only on the whole surface of the blastomeres but also on the protoplasmic bridge connecting them, and on the protoplasmic drops of the broken bridge. A similar condition is observable in the eggs of *Strongylocentrotus pulcherrimus*. As mentioned above, the blastomeres were covered with the radial halo-layer at all times when the egg was developed in calcium-free sea water. And if it is possible to assume that the distribution of the radial halo-layer is restricted only on the old surface of the egg, the presence of this layer on the side of the bridge attachment of the blastomeres must be a proof showing the extension of the cortical cytoplasm during the course of cleavage in calcium-free sea water.

SUMMARY

1) When the fertilized eggs of *Strongylocentrotus pulcherrimus* are cultured in calcium-free sea water the blastomeres separate widely, being connected with a long protoplasmic bridge.

2) The whole surface of the blastomeres is covered at all times of the first cleavage in calcium-free sea water with pigmented cortical cytoplasm. This shows the extension of the old surface for covering the increased surface area.

3) Presence of the radial halo-layer was observed.

4) The pigment granules in the eggs of this species are not identical with the osmium-blackened granules which are a kind of endoplasmic granule.

5. The cleavage picture characteristic of the calcium-free sea water could be obtained only when the eggs were washed many times with this artificial medium.

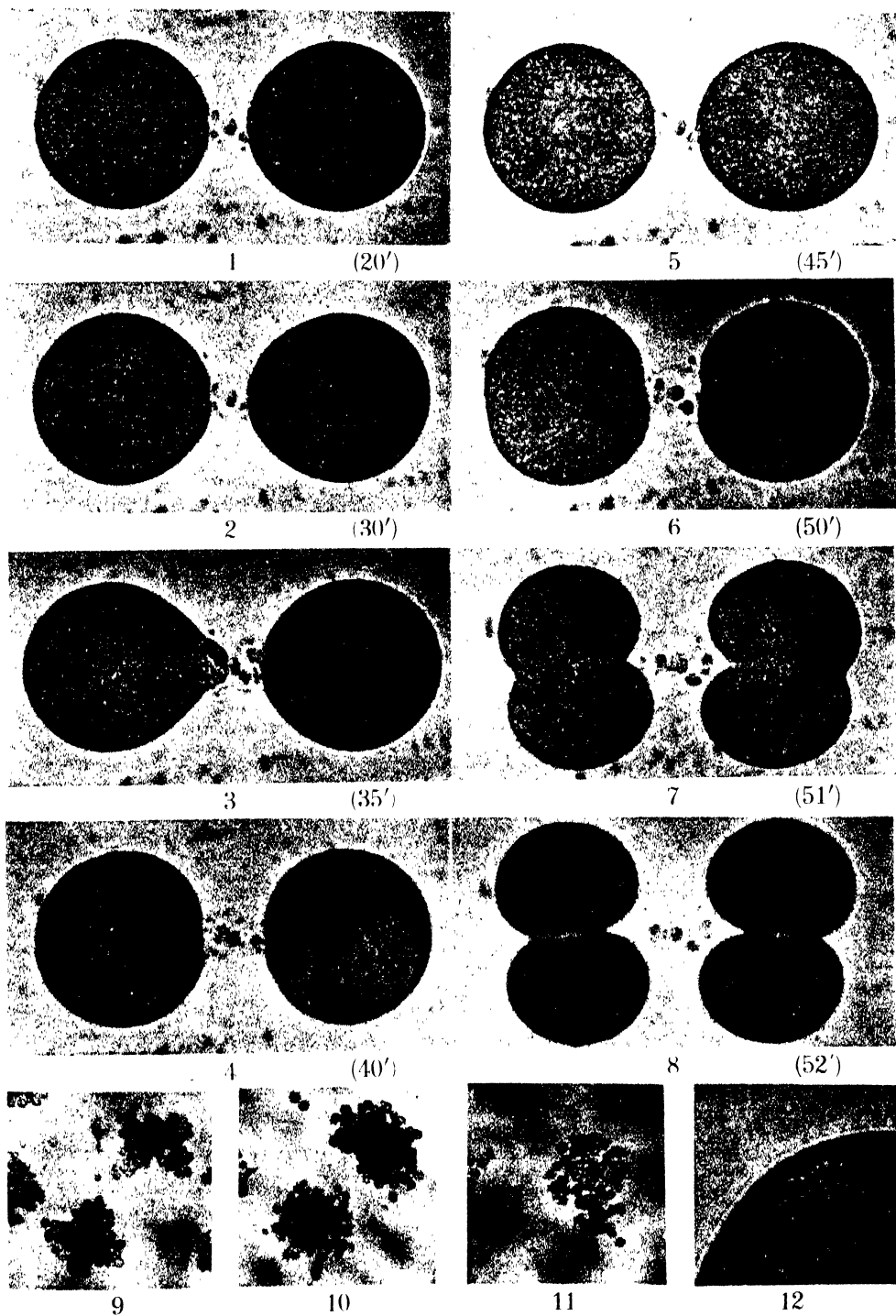
LITERATURE CITED

- DAN, K., J. C. DAN T. YANAGITA, 1938. Behavior of the cell surface during cleavage. II. *Cytologia* 8: 521-531.
- DAN, K. and J. C. DAN, 1940. Behavior of the cell surface during cleavage. III. On the formation of new surface in the eggs of *Strongylocentrotus pulcherrimus*. *Biol. Bull.* 78: 486-501.
- HERBST, C. 1900. Über das Auseinandergehen von Furchungs- und Gewebszellen in kalk-freien Medium. *ROUX' Arch.* 9: 424-463.
- MOTOMURA, I. 1935. Determination of the embryonic axis in the eggs of Amphibia and Echinoderms. *Sci. Rep. Tohoku Imp. Univ. Ser. Biol.* 10: 211-245.
- MOTOMURA, I. 1936. On the cytoplasmic structure of the egg of a sea urchin, *Strongylocentrotus pulcherrimus*, from the view point of embryology. *Zool. Mag.* 48: 89-97. (in Japanese)

EXPLANATION OF PLATE XVIII

Figs. 1-8. First cleavage of an egg of *Strongylocentrotus pulcherrimus* in calcium-free sea water showing the presence of the complete ring of the pigmented cortical cytoplasm. The egg was washed 15 times with calcium-free sea water before the beginning of the first cleavage, and then sealed in a depression slide. $\times 290$. Fig. 1. 20 minutes after the appearance of the constriction of the first cleavage furrow. The protoplasmic bridge has been broken down into drops of protoplasm. Fig. 2. 30 minutes after. Fig. 3. 35 minutes after, showing the amoeboid movement of the cytoplasm at the side of the bridge attachment. Fig. 4. 40 minutes after, showing the formation of clusters of the pigmented cortical cytoplasm at the side of the bridge attachment of the blastomeres. Fig. 5. 45 minutes after, showing the complete ring of the pigmented cortical cytoplasm in an optical section of the blastomeres. Fig. 6. 50 minutes after. Beginning of the second cleavage, showing equal thickness of the cortical cytoplasm. Fig. 7. 51 minutes after. Fig. 8. 52 minutes after. The egg was photographed with a tricolor blue filter, showing the presence of the pigmented cortical cytoplasm on the whole surface of the blastomeres.

Figs. 9-11. Eggs in calcium-free sea water photographed 8 hours after fertilization showing the effect of the number of washings with calcium-free sea water. $\times 60$. Fig. 9. Eggs washed three times with calcium-free sea water. Blastomeres cling together to form compact masses. Fig. 10. Eggs washed 6 times. Fig. 11. Egg washed 15 times showing the absence of the capacity of clinging in the blastomeres. Fig. 12. Radial halo-layer on the surface of the fertilized egg, which was washed many times with calcium-free sea water. Photographed in the same artificial medium. The egg was at the beginning of the first cleavage. $\times 730$.



EMBRYOLOGICAL OBSERVATIONS ON TAIWANIA CRYPTOMERIOIDES HAYATA

By

YOSINORI SUGIHARA

Biological Institute, Tōhoku Imperial University, Sendai

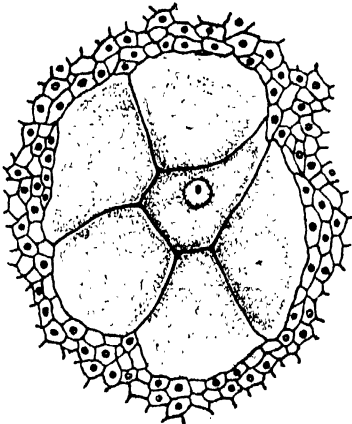
(With Plates XIX, XX and 2 text-figures)

(Received February 7, 1941)

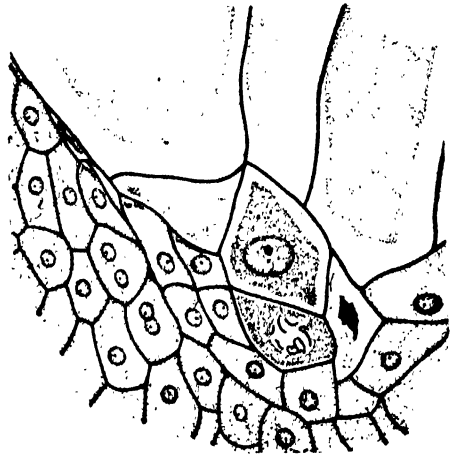
Taiwania is a monotypic genus belonging to the family *Taiwaniaceae*. This genus was established in 1906 by the investigation of the late Professor B. HAYATA. Up to the present, only Formosa, Yunnan and Burma are enumerated as the native places of this plant. The chromosome number of this plant was estimated by MATSUMOTO ('33) and by SAX and SAX ('33) to be twenty-two in the diploid generation, but the embryological study has not been undertaken yet. To make a collection of the material for the embryological studies of this plant the present writer stayed last year (1940) from April to August in Formosa. The material was collected exclusively in the neighbourhood of a village called Sin-Taiheizan, situated in the north-east part of the island. This village has an altitude of about 1900 m. It is cool and foggy in summer and snows lightly in winter. In the vicinity of the village *Taiwania cryptomerioides* grows here and there among the forest of conifers, mainly consisting of *Chamaecyparis taiwanensis* MASAMUNE et SUZUKI and *Ch. formosensis* MATSUMURA. As for *Taiwania cryptomerioides* only large trees were found with cones. Moreover the fertility of the cone is generally very low. Under these conditions the collection of the materials was somewhat harder than had been expected. The cones were obtained only from the spots where large trees had been cut down for forestry purposes. The methods used for the observations are the same as those which the writer adopted in the previous investigations (SUGIHARA '39, '41). But in the fixations the hand pump was usually used to make the penetration of the fixative (chromo-acetic solution) more rapid. The observation concerning the suspensor formation was done in a forestry station in Sin-Taiheizan, using fresh materials.

In the male gametophyte of *Taiwania cryptomerioides* the body cell divides immediately before the fertilization. As a result of the mitosis

two sperm cells are formed which are alike in size and shape (Pl. XIX Figs. 1, 2). The female prothallium is very small in size. The archegonia are grouped together in a complex at the apex of the prothallium but the complex has no sterile tissue at the centre which is found in *Cunninghamia* (MIYAKE '10) (Text-fig. 1). The archegonia of a complex are generally four to nine in number. The wall of the mature archegonium is very thick. In rare instances, an archegonium or a complex of several archegonia are found additionally at the lateral or the basal part of the prothallium. The jacket layer of the archegonial complex is not clearly differentiated but sometimes several cells containing dense cytoplasm are found on the boundary of the complex (Text-fig. 2).



Text-fig. 1. Cross-section of an archegonial complex. $\times 210$.



Text-fig. 2. Jacket cells at the basal part of an archegonial complex. $\times 370$.

In 1940, the fertilization took place in the middle of July. The contact of the female and the male nuclei is carried out at the upper part of the archegonium and then they pass down towards the bottom of the archegonium (Pl. XIX Figs. 3, 4). In this respect *Taiwania* agrees with COKER's description on *Taxodium* ('03), but not with LAWSON's on *Cryptomeria* ('04). The contacted nuclei are wrapped with granular cytoplasm. At the base of the archegonium the fertilized nucleus carries out proembryonal mitoses three times and eight nuclei are formed (Pl. XIX Figs. 5-8). Then the wall-formation takes place between the eight nuclei and two tiers of cells are formed, five or six cells being found in the upper and two or three in the lower tier (Pl. XIX Fig. 8). But the

upper tier is open to the archegonial cavity, as is usual in most conifers. In the next stage, probably by the division of the upper tier, three tiers of cells are formed. In most conifers the uppermost tier in such a stage has no wall towards the general cytoplasm of the archegonium, but in *Taiwania* this tier has complete walls in all sides (Pl. XIX Fig. 9). Later in this tier, namely the rosette tier, the divisions of the cells occur irregularly. These conditions are not observed either in *Taxodium* or *Cryptomeria*. Meanwhile, the cells of the middle tier of the proembryo elongate windingly as the prosuspensor (Pl. XX Figs. 12, 15, 19). As far as the writer's observation is concerned, the separation of the prosuspensor cells does not take place at least in the early stages. Later, however, the tips of the prosuspensor cells become slightly separated from each other. In this respect *Taiwania* resembles *Taxodium* rather than *Cryptomeria*. Concerning this point in connection with *Cryptomeria*, BUCHHOLZ ('31) writes as follow: "There are usually four or five elongating cells, which soon become separated into individual prosuspensor cells bearing the separated embryonic cells at their lower ends."

The cells in the lowest tier of the proembryo are the embryonic units (Pl. XX Figs. 13, 14). In a later stage of development, however, sometimes some of them become degenerated (Pl. XX Fig. 16). The embryonic units at the tip of the prosuspensor divide now independently to form separate embryos (Pl. XX Figs. 16-18). Then the cells situated next to the prosuspensor elongate as the embryonal tubes, thus the primary suspensor is not formed in this plant (Pl. XX Fig. 19). In short, cleavage polyembryony occurs regularly in *Taiwania cryptomerioides*.

Fortunately the writer was able to count the chromosome number of this species in the female prothallial cells (Pl. XIX Figs. 10, 11). It was eleven. Thus the writer's estimation agrees well with that of MATSUMOTO ('33) and SAX and SAX ('33). In some female prothallia of the post-fertilization stages bi-, tetra- or polynucleate cells were observed and sometimes in the mitoses of these cells nearly diploid or triploid number of chromosomes was counted.

SUMMARY

1) In *Taiwania cryptomerioides* the two sperm cells formed by the division of a body cell are alike in size and shape.

2) Archegonia are grouped together in a complex at the prothallial apex.

3) In 1940, the fertilization occurred in the middle of July in Sin-Taiheizan, Formosa.

4) The first division of the proembryo takes place at the bottom of the archegonium and the wall-formation of the proembryo occurs after the third mitosis.

5) The components of the embryo system are the rosette cells, the prosuspensor, the embryonal tubes and the embryo proper. The primary suspensor is not formed.

6) Conspicuous cleavage polyembryony regularly occurs.

7) The chromosome number is eleven in the haploid generation.

Here the writer wishes to express his cordial thanks to Professor Dr. M. TAHARA for his kind suggestions and criticisms during the course of this investigation. Thanks are also due to Professor Dr. S. HIBINO, Professor Dr. G. MASAMUNE, Mr. H. KITUKAWA, Mr. T. SÔMA, Mr. H. YAMADA and Mr. A. SIMOMURA who took a kindly interest in collecting the materials in Formosa.

The present work was aided by a grant from the Japan Society for the Promotion of Scientific Research to which the writer wishes to express his sincere thanks.

LITERATURE

- BUCHHOLZ, J. T., 1931. The Suspensor of *Cryptomeria japonica*. Bot. Gaz., **93**: 221-226.
- COKER, W. C., 1903. On the Gametophyte and Embryo of *Taxodium*. Bot. Gaz., **36**: 1-27, 114-140.
- LAWSON, A. A., 1904. The Gametophytes, Fertilization and Embryo of *Cryptomeria japonica*. Ann. Bot., **18**: 417-444.
- MATSUMOTO, K., 1933. Über die Chromosomenzahl von *Cryptomeria japonica* D. DON und *Taiwania cryptomerioides* HAYATA. Bot. and Zool., **1**: 1751-1756. (Japanisch mit deutscher Zusammenfassung).
- MIYAKE, K., 1910. The Development of the Gametophytes and Embryogeny in *Cunninghamia sinensis*. Beih. Bot. Centbl., **27**: I: 1-25.
- SAX, K. and H. J. SAX, 1933. Chromosome Number and Morphology in the Conifers. Journ. Arnold Arb., **14**: 356-375.
- SUGIHARA, Y., 1939. Embryological Observations on *Thujaopsis dolabrata* var. *Hondai* MAKINO. Sci. Rep. Tôhoku Imp. Univ., 4th Ser., Biol., **14**: 291-303.
- SUGIHARA, Y., 1941. The Embryogeny of *Cunninghamia lanceolata* HOOKER. Sci. Rep. Tôhoku Imp. Univ., 4th Ser., Biol., **16**: 187-192.

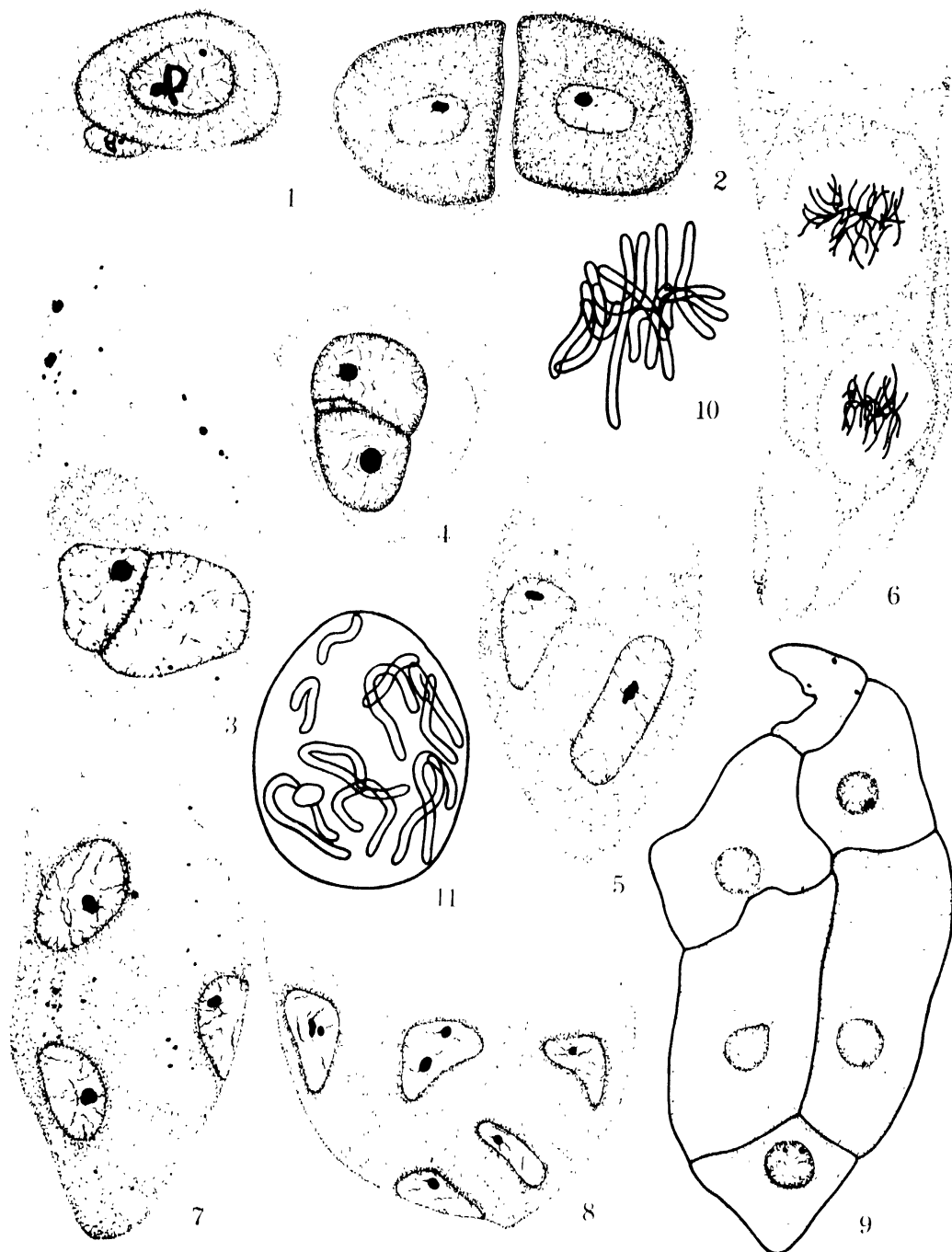
EXPLANATION OF PLATES

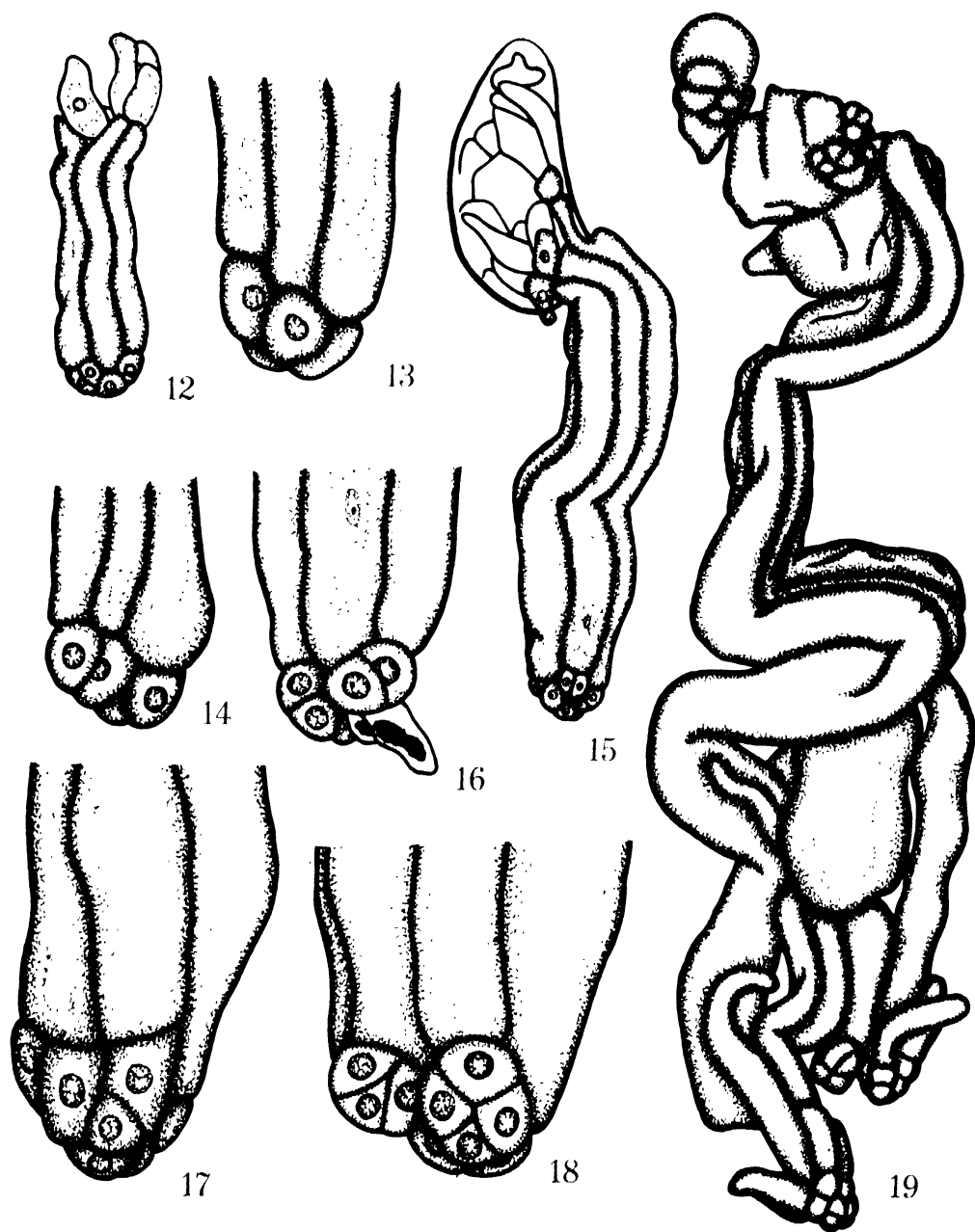
Plate XIX.

- Fig. 1. A body cell. $\times 660$.
Fig. 2. Two sperm cells formed by the division of a body cell. $\times 660$.
Figs. 3-4. Fertilization. $\times 660$.
Fig. 5. Two nucleus stage of a proembryo. $\times 660$.
Fig. 6. Proembryonal second division in metaphase. $\times 660$.
Fig. 7. Four nucleus stage of a proembryo. $\times 660$.
Fig. 8. Eight nucleus stage in which wall-formation is taking place. $\times 660$.
Fig. 9. Three tier stage of a proembryo. $\times 660$.
Figs. 10-11. Mitosis in a prothallial cell. Eleven chromosomes are clearly counted. $\times 2280$.

Plate XX.

- Fig. 12. Early stage of the prosuspensor elongation. $\times 130$.
Figs. 13-14. Embryonic units at the tip of a prosuspensor in the early stage. $\times 280$.
Fig. 15. More advanced stage of the prosuspensor elongation. Each embryonic unit is divided once. $\times 130$.
Figs. 16-17. The same in a larger magnification. $\times 280$.
Fig. 18. More advanced stage of the embryonic units at the tip of a prosuspensor. $\times 280$.
Fig. 19. Whole embryo showing the elongation of the embryonal tubes. $\times 130$.





DEVELOPMENT OF THE EMBRYO-SAC IN *STATICE* *JAPONICA* SIEBOLD ET ZUCCARINI

By

MASA-O YA-E

Biological Institute, Tôhoku Imperial University, Sendai

(With 20 Text-figures)

(Received February 8, 1941)

As to the development of the embryo-sac of the genus *Statice*, DAHLGREN (1916) reported that in *Statice bahusiensis* FR., *S. Gmelini* WILLD., *S. macropetra* WEBB. et BERTH. and *S. sinuata* L., it was of the *Adoxa*-type (*Lilium*-type); but FAGERLIND (1938, 1939) reported that in a species belonging to the section *Eu-Limonium*, it was of the *Penaea*-type, and in *S. Bonduelli* LESTIB., *S. sinuata* L. *rosea* and *S. Suworowii* RGL., it was of the *Fritillaria*-type. The present writer investigated the embryo-sac development of *Statice japonica* SIEBOLD et ZUCCARINI. The results obtained will be described below.

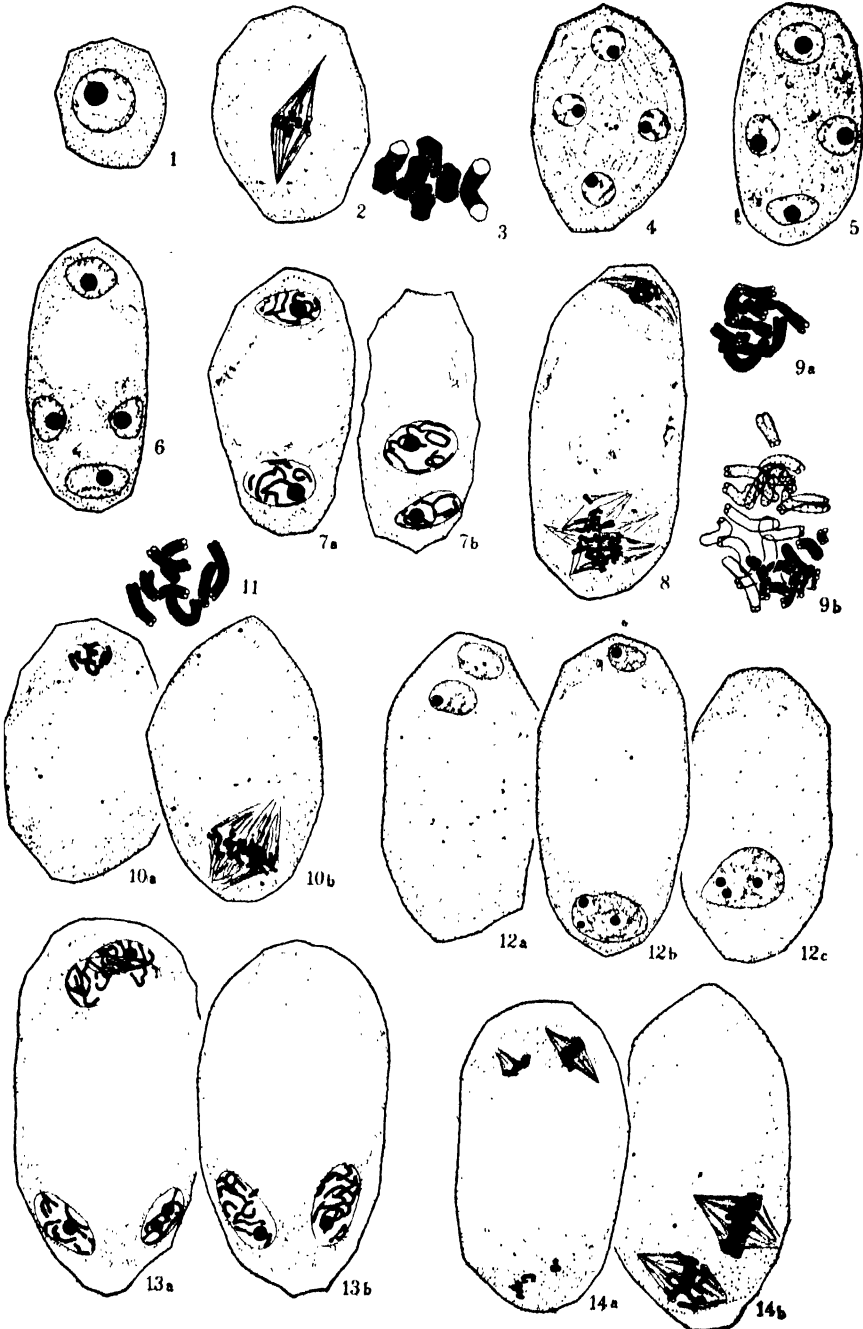
MATERIAL AND METHOD

Statice japonica growing on the seashore at Nobiru-Mura, Miyagi-Ken was collected on May 17, 1939 and was cultured in pots. The material was fixed on August 11 and 12 by NAVASHIN's solution. The material in the solution was first placed 7-10 minutes under a vacuum pump. The sections, 10-20 microns thick, were stained in Heidenhain's iron-alum haematoxylin.

OBSERVATIONS

The embryo-sac mother cell just before the meiosis is nearly globular (Fig. 1), but soon becomes ellipsoidal. In the metaphase of the heterotypic division, eight gemini were observed (Fig. 2, Fig. 3).

The four megasporial nuclei are formed as the result of the meiosis, but they are not separated by a wall, thus presenting the first four nucleate stage of the embryo-sac development. The four nuclei, which are similarly small and spherical, occupy positions corresponding to the four points of a diamond, fibrous plasma connecting these four nuclei (Fig. 4). Meanwhile the embryo-sac elongates and the four nuclei grow somewhat



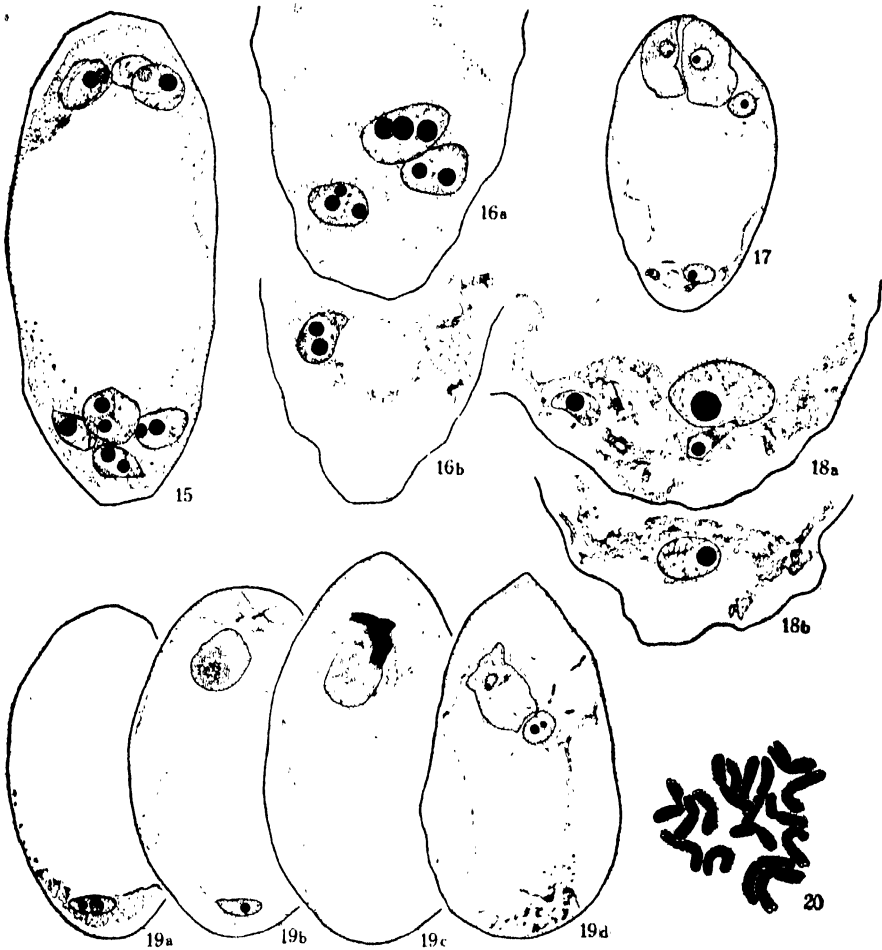


Fig. (1). The embryo-sac mother cell. (2). The metaphase of the heterotypic division. (3). The same in a larger magnification. (4), (5) and (6). The first four nucleate stage. (7a) and (7b). The prophase of the third division in two successive sections. (8). The metaphase of the third division. The chalazal three mitotic figures do not still unite. (9a) and (9b). The same in a larger magnification. (9a) The equatorial plate at the micropylar end. (9b). The equatorial plate in the chalazal region. (10a) and (10b). The metaphase of the third division. Three chalazal mitotic figures are already united. (a) and (b) are the two successive sections. (11). The same as Fig. 10a in a larger magnification. (12a), (12b) and (12c). The second four nucleate stage in three successive sections. (13a) and (13b). The prophase of the fourth division, in two successive sections. (14a) and (14b). The metaphase of the fourth division in two successive sections. (15). The early eight nucleate stage. (16a) and (16b). The chalazal nuclei in a later stage. (a) and (b) are the two successive sections. (17). The finished embryo-sac. One synergid and one antipodal nucleus do not appear in this figure. (18a) and (18b). The chalazal region of the same in a larger magnification. (a) and (b) are the two successive sections. (19a),

irregular in shape. The two nuclei in the midregion begin to migrate towards the nucleus lying in the chalazal region (Fig. 5). The cytoplasm becomes now considerably vacuolated. The four nuclei continue to enlarge and the lower three come close together in the chalazal region. Even in this condition the four nuclei are all alike in size and shape. (Fig 6).

After this polarization, the four nuclei still enlarge and enter simultaneously into the prophase of the third division. Eight slender chromosomes can be seen in each nucleus. Small vacuoles in the cytoplasm become united to form a large central vacuole (Fig. 7a and b). In the metaphase, at the micropylar end eight chromosomes and in the chalazal region three mitotic figures lying close together are observed. Without difficulty eight chromosomes are counted in each of these three mitotic figures in the chalazal region. After a while these three unite to form a single triploid equatorial plate (Fig. 8, Fig. 9a and b, Fig. 10b).

By the third division the second four nucleate stage of the embryo-sac development is reached. The two haploid nuclei in micropylar region are small and contain one nucleolus, while the two triploid nuclei in the chalazal region are much voluminous and contain three nucleoli (Fig. 12a, b and c). These three nucleoli, however, usually unite and only one or two nucleoli are formed in each nucleus.

The four nuclei simultaneously proceed next to the prophase of the fourth division (Fig. 13a and b). The one micropylar nucleus of the embryo-sac shown in Fig. 13a is much larger than the others, although we cannot offer any reasonable explanation for this phenomenon. In the metaphase of this division, the two small micropylar and the two large chalazal spindles are all typical in every respect (Fig. 14a and b).

By this fourth division viz. the last division, the eight nuclei are produced. Peculiarly they do not show any marked difference in size (Fig. 15). The embryo-sac now becomes much enlarged and in the micropylar region one egg-cell, two synergids and one polar nucleus, and in the chalazal region three antipodal nuclei and one polar nucleus, are organized (Fig. 17). Thus, in short, the embryo-sac development of

(19b), (19c) and (19d). The embryo-sac soon after fertilization in four successive sections. (19a). A small antipodal nucleus and a larger chalazal polar nucleus the half of which is shown in (b) (19b). The egg cell in mitosis. (19c). The synergid broken by the pollen tube. (19d). Another synergid and the large micropylar polar nucleus. (20). The mitosis in the egg cell in a larger magnification. *Magnification*: (1), (2), (4), (5), (6), (7), (8), (10), (12), (13) and (14). $\times 725$. (3), (9) and (11). $\times 1520$. (15), (16) and (18). $\times 725$. (20). $\times 1520$. (17) and (19). $\times 225$. All the figures are so arranged that the micropylar end is towards the top of the figures.

Statice japonica is of the *Fritillaria*-type.

In Fig. 15 the four nuclei in the chalazal region are all alike. But in Fig. 16a, the uppermost nucleus is much larger than the others and seems to be destined to become the chalazal polar nucleus.

The later appearance of the chalazal region is shown in Fig. 18a and b, where the two antipodal nuclei (Fig. 18a) are rather small and are irregular in shape, showing a sign of degeneration. In the finished embryo-sac, usually none or only one antipodal nucleus, is seen. The migration of the chalazal polar nucleus towards the micropylar pole nucleus does not occur in this species.

An embryo-sac immediately after fertilization is presented in Fig. 19, where the diploid egg nucleus in mitosis with sixteen chromosomes is shown (Fig. 19b, Fig. 20). In this embryo-sac, the one synergid is broken by the pollen tube (Fig. 19c), and the other is beginning to degenerate (Fig. 19d).

DISCUSSION

FAGERLIND (1939) investigated *Statice Bonduelli*, *S. sinuata rosea* and *S. Suworowii*. According to his observations the embryo-sac development of these plants is of the *Fritillaria*-type as in *S. japonica*. In his paper FAGERLIND reports that in these three species the three chalazal megasporial nuclei are smaller than the one remaining in the micropylar region, and in *S. Bonduelli* and *S. sinuata rosea*, this difference is especially remarkable. In *S. japonica* investigated by the present writer, the nuclei in both regions are nearly alike in size (Fig. 6).

FAGERLIND (1939) also says that in *S. Bonduelli* and *S. sinuata rosea*, the development of the chalazal region after the third nuclear division is extremely irregular and in the newly organized embryo-sac, the number of the chalazal nuclei is one, two or three and they are very unequal in size and shape. FAGERLIND considers that this irregularity is caused by the omission of the fourth division, the unequal distribution of the chromosomes, the union of only two of the three chalazal megasporial nuclei, or the union of some of the nuclei which already carried out the third division. According to his description, however, in *S. Suworowii*, the chalazal polar nuclei develop normally, and three antipodal cells and a chalazal polar nucleus are formed. In *S. japonica*, the present writer also observed the normal development of the chalazal nuclei. But in this species the antipodal cells are not formed, the nuclei always remaining

free (Fig. 17, Fig. 18a and b).

Concerning the mitosis during the embryo-sac development of *Statice*, up to the present only the prophase of the third division has been observed by FAGERLIND (1939) in *S. Bonduelli* and *S. sinuata rosea*, so the present writer's observation of the metaphase of the meiosis and the metaphase of the third and the fourth division surely gives exact knowledge with reference to the embryo-sac development of this genus.

As to the degeneration of the chalazal nuclei, FAGERLIND (1939) reports its rather early commencement in the three species investigated by him, and in *S. Bonduelli* and *S. sinuata rosea*, sign of it often already appears at the time of the third division. In *S. japonica*, however, the degeneration of the chalazal nuclei first begins after the nearly perfect completion of the embryo-sac development.

In 1916 DAHLGREN reported in *S. bahusiensis* that he observed the contact of the two polar nuclei after fertilization. As above mentioned, the present writer did not observe such phenomenon in *S. japonica*. In this plant, after fertilization the micropylar polar nucleus, perhaps diploid in its constitution, contains a large and a small nucleolus (Fig. 19d).

As to the chromosome number of *Statice*, in 1930 ALESKOWSKY reported eighteen and its multiples in somatic cells of four species and one variety, and in 1937 WULF confirmed that in three species the basic chromosome number of this genus is nine. However, SUGIURA investigated (1936, 1939), ten species, two of which had been formerly investigated by WULF (1937), and reports that the basic chromosome number of this genus is eight. The chromosome number of *S. japonica* had never been studied before. The writer's present investigation gives a strong affirmation to SUGIURA's estimation.

SUMMARY

- 1 The development of the embryo-sac of *Statice japonica* SIEBOLD et ZUCCARINI was investigated.
2. The development is of the *Fritillaria*-type.
3. The mitosis during the embryo-sac development was exactly observed.
4. The union of the three megasporial nuclei in the chalazal region occurs in the metaphase of the third division.
5. The four megasporial nuclei are nearly alike in size.
6. In the chalazal region the fourth mitosis is carried out in a typical manner. The degeneration of the nuclei in this region takes place some-

what later than in the other species of this genus formerly investigated by the other authors. The antipodal nuclei remain free, without forming cells. The chalazal polar nucleus does not unite with the micropylar one.

7. The chromosome number of *S. japonica* is eight in the haploid and sixteen in the diploid nucleus.

This work has been carried out with the help of the valuable suggestions and criticisms of Professor Dr. M. TAHARA, to whom the writer takes great pleasure in expressing his hearty thanks.

LITERATURE CITED

- DAHLGREN, K. V. O. 1916. Zytologische und embryologische Studien über die Reihen Primulales und Plumbaginales. Kun. Svenska Vet. Akad. Handl. 56, p. 1-80.
- FAGERLIND, F. 1938. Wo kommen tetrasporische durch drei Teilungsschritte vollentwickelte Embryosäcke unter den Angiospermen vor? Bot. Not. 1938, p. 461-498.
- FAGERLIND, F. 1939. Drei Beispiele des *Fritillaria*-type. Svensk Bot. Tidskrift 33, p. 188-204.
- SUGIURA, T. 1936. Studies on the chromosome number in higher plants, with special reference to cytokinesis. 1. Cytologia 7, p. 544-595.
- SUGIURA, T. 1939. Chromosome number in Plumbaginaceae. Cytologia 10, p. 73-76.
- TISCHLER, G. 1937. Pflanzliche Chromosomen-Zahlen. Tabulae Biologicae 12, p. 73-76.
- WULF, H. D. 1937. Karyologische Untersuchungen an der Halophytenflora Schleswig-Holsteins. Jahrb. f. wiss. Bot. 84, p. 812-840.

ÜBER DAS REDOXPOTENTIAL DES PRESSSAFTES EINER ETIO- LIERTEN PFLANZE, *VICIA FABA*, UND DIE POTENTIAL- VERÄNDERUNGEN EINIGER SUBSTRATE UNTER DER EINWIRKUNG DES VON DER BETREFFENDEN PFLANZE EXTRAHIERTEN ENZYMS

VON

TOMIO KAKUKAWA

Biologisches Institut der Kaiserlichen Tōhoku Universität, Sendai

(Mit 7 Figuren)

(Eingegangen am 13 Februar, 1941)

I. EINLEITUNG

In den letzten Jahren ist eine Anzahl Untersuchungen über das Redoxpotential der Mikroorganismen gemacht worden, und zwar betreffs der Bakterien von QUASTEL, STEPHENSON und WHETHAM ('25), HEWITT ('30), KUSNETZOW ('31), ELEMA, KLUYVER und VAN DALESSEN ('34), KORR ('35), KLUYVER und HOOGERHEIDE ('36) u. s. w. und betreffs der Hefen von CANNAN, COHEN und CLARK ('26), AUBEL, AUBERTIN und GENEVOIS ('29), BOYLAND ('30), KNIGHT ('30), LEHMANN ('34), MICHAELIS und SMYTHE ('36), FROMAGEOT und DESNUELLE ('35), FROMAGEOT und BOST ('37), LIPMANN ('33 und '36), KLUYVER und HOOGERHEIDE ('34 und '36), KAKUKAWA ('38), GAUTHERET ('39) u. s. w. Dank diesen Untersuchungen ist es jetzt klar geworden, dass das Redoxpotential mit der Natur der in den Zellen vor sich gehenden Stoffwechselprozesse in einer näheren Beziehung steht.

Auch bei anderen Pflanzen ist eine Reihe von Untersuchungen in dieser Richtung durchgeführt worden. BROOKS ('26) bestimmte rH des Zellsaftes und des Protoplasmas in *Valonia*. RAPKINE und WURMSER ('26) mass rH in den Zellen von *Spirogyra*. TANG und LIN ('36) untersuchte das Redoxpotential der *Chlorella*-Suspension im Licht und im Dunkel. Was die höheren Pflanzen anbelangt, so mass MAYEK und PLANTEFOL ('28) das Redoxpotential der Presssäfte von verschiedenen Pflanzengeweben mit Hilfe der Redoxindikatoren. KRASSINSKY ('35) bestimmte die Redoxpotentiale der Reservestoffbehälter: Kartoffelknollen, Zwiebeln und Rüben. WARTENBERG und Mitarbeiter ('35 und '36) führten die Versuche über

das Redoxpotential der Kartoffelknollen aus. MARCKS FRANKE ('37) bestimmte die Redoxpotentiale der Presssäfte von gesunden und viruskranken Pflanzen, *Nicotiana*, *Beta* und *Phaseolus*. KAUSCHE ('37) untersuchte auch das Redoxpotential der Presssäfte von viruskranken und gesunden Tabakpflanzen. ROSS ('38) mass die Redoxpotentiale der chlorotischen und grünen Maispflanzen.

Wenn aber die eingehende Untersuchung bei höheren Pflanzen in dieser Richtung geht, so stösst man immer auf die Schwierigkeit, welche darin liegt, dass sie im Gegensatz zur Untersuchung von Mikroorganismen nicht imstande ist, das Redoxpotential der Zellen oder Gewebe der höheren Pflanzen unter den natürlichen Bedingungen zu bestimmen. Aus diesem Grunde versteht es sich von selbst, dass so weitgehende Untersuchungen wie bei den Mikroorganismen bei den höheren Pflanzen noch wenig ausgeführt sind. Aber es ist sehr wünschenswert, die nähere Beziehung zwischen dem Redoxpotential und dem in den Zellen vor sich gehenden Stoffwechsel auch bei den höheren Pflanzen weiter zu verfolgen. Von diesem Standpunkt aus versuchte ich zunächst das Redoxpotential der höheren Pflanzen mit Hilfe der potentiometrischen Methode zu bestimmen, obwohl es hierbei mehr oder weniger grosse Schwierigkeiten gibt. Dabei muss der Presssaft unvermeidlich benutzt werden. Wenn es auch der potentiometrischen Methode nicht gelang, das Redoxpotential unter der intakten Bedingung zu bestimmen, ist ihr doch der Vorteil zuzuschreiben, dass sie nicht nur den erweiterten Potentialbereich verwirklichen kann, sondern auch von den bei der Indikatormessung herbeigeführten Einwänden, d. h. der giftigen und katalytischen Wirkung, dem Beschwerungs- und dem Kapazitätsfaktor und der Nichtübereinstimmung der Werte bei verschiedenen Indikatoren an demselben Potentialwert, frei ist.

Der Bereich dieses Versuchs ist darauf beschränkt, dass lediglich das Redoxpotential des Presssaftes von einer einzigen Pflanze, *Vicia Faba*, bestimmt wird, ohne Eintreten in die Untersuchung der Beziehung zwischen dem Redoxpotential und dem Stoffwechsel der betreffenden Pflanze.

II. METHODIK

Als Untersuchungsmaterial kam die etiolierte Pflanze, *Vicia Faba*, welche in Töpfen unter schwarzen Decken drei bis vier Wochen lang heranwuchs, zur Verwendung, in der Hoffnung, dass die der Elektrode bewirkenden Redoxsysteme, welche sich in den Pflanzengeweben befinden, durch die Entfernung der physiologisch und chemisch komplizierten

Photosynthese, welche durch das Vorhandensein des Chlorophylls hervorgerufen wird, möglichst einfach bleiben können.

Der Presssaft wurde aus Stengeln der betreffenden Pflanze mit Hilfe der Handpresse hergestellt, durch ein weitmaschiges Gasetuch filtriert und sofort zentrifugiert, um kleine Stückchen der Gewebe zu separieren. Um den bei der Herstellung des Presssaftes eindiffundierenden Sauerstoff vermeiden zu können, soll dieses Verfahren so schnell als möglich vollbracht und dabei die Saftoberfläche mit Paraffinöl bedeckt werden. Danach wurde sofort der oberklare Presssaft in drei etwa 6 ccm haltige Elektrodengefässe eingesetzt, mit der allergrössten Sorgfalt darauf, dass gar kein Luftbläschen darin bleiben soll.

Ein Elektrodengefäss wurde ausser mit einer blanken Platinelektrode, mit deren Hilfe das Potential gegenüber der gesättigten KCl-Kalomel-elektrode bestimmt wurde, einem KCl-Kapillargarheber zur Elektrolytverbindung, und einem Glasrohr ausgerüstet, dessen Hahn nach der Ausdehnung des Inhaltes bei der Erwärmung bis zu 30°C verschlossen wurde, wodurch der Presssaft im Gefäss völlig von der Luft abgesondert werden konnte. Alle KCl-Kapillargarheber wurden in ein zentrales Gefäss mit gesättigter KCl-Lösung gemündet. Die so ausgerüsteten drei Elektrodengefässe wurden im Wasserthermostat (30°C) aufgestellt.

Zur Messung des Potentials wurde die Kompensationsmethode benutzt, und der Messapparat bestand aus einem Kapillarelektrometer als Nullinstrument und einem Messbrückenpotentiometer. Das besondere Verfahren, womit einige Versuche ausgeführt werden, sei dementsprechend in den betreffenden Abschnitten erwähnt.

Schliesslich sei noch bemerkt, dass die im folgenden wiedergegebenen Potentialwerte alle auf die Normalwasserstoffelektrode bezogen sind.

III. ERGEBNISSE

1. Die Wasserstoffionenkonzentration des Presssaftes von *Vicia Faba*.

Vor der Bestimmung des Redoxpotentials des Presssaftes muss man zuerst die Aufmerksamkeit darauf richten, dass das Redoxpotential bekanntlich nicht nur durch das Verhältnis der Redoxphasen, sondern auch durch die Wasserstoffionenkonzentration bedingt wird. Es muss deshalb in Betracht kommen, ob der Presssaft das Puffervermögen der Wasserstoffionenkonzentration besitzt. Um diese Frage klarzustellen habe ich den folgenden Versuch ausgeführt.

Der Presssaft wurde sofort nach dessen Herstellung in ein dickes

Reagensrohr eingebracht und dann im Thermostat (30°C) aufgestellt. Dabei wurde das Paraffinöl auf die Oberfläche des Presssaftes versetzt, um die direkte Berührung mit der Luft zu vermeiden. Zur Bestimmung der Wasserstoffionenkonzentration wurde die MICHAELISSche Wasserstoffelektrode, in welche der oben genannte Presssaft von dem betreffenden Rohr aus einpipettiert wurde, verwendet, wobei als Standardflüssigkeit die Acetatlösung (pH=4,62) benutzt wurde.

Die erhaltenen Ergebnisse sind in Tabelle 1 zusammengestellt und in Abb. 1 graphisch wiedergegeben.

TABELLE 1.
Wasserstoffionenkonzentration des Presssaftes von
Vicia Faba. Bei 30°C.

Zeit in Std.	pH-Wert	Zeit in Std.	pH-Wert
0	6,03	8	5,91
1	6,02	9	5,74
2	6,00	10	5,63
3	5,96	11	5,48
4	5,95	12	5,35
5	5,95	13	5,26
6	5,93	—	—
7	5,92	24	5,05

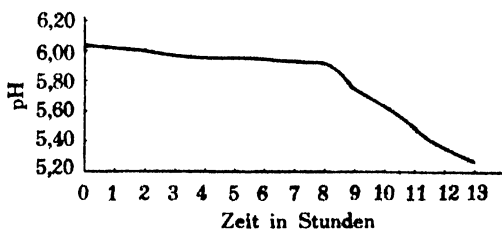


Abb. 1. pH-Zeitkurve des Presssaftes von *Vicia Faba*.

Aus diesen Ergebnissen geht hervor, dass im Verlauf der ersten drei Stunden nur geringe Abnahme des pH-Wertes, etwa 0,07 Einheit, auftritt und danach ziemlich lange Zeit darauf konstant bleibt. Erst nach etwa 9 Stunden findet die Abnahme des pH-Wertes verhältnismässig schnell statt. Daraus lässt sich nun entnehmen, dass die Wasserstoffionenkonzentration des Presssaftes, welcher im Wasserthermostat (30°C) unter Aussperrung der direkten Berührung mit der Luft hergestellt wurde, ziemlich lange Zeit um pH=5,95 gleichsam gepuffert wird, abgesehen von der späteren Abnahme.

Auf Grund dieser Ergebnisse scheint es nicht gewagt anzunehmen, dass das Redoxpotential des Presssaftes beim hier gefundenen pH-Bereich ohne Rücksicht auf die pH-Veränderung bestimmt werden kann.

2. Das Redoxpotential des Presssaftes von *Vicia Faba*.

An erster Stelle wurde das Redoxpotential des betreffenden Presssaftes potentiometrisch bestimmt, wobei es sich von selbst verstand, dass man den Presssaft im Elektrodengefäß nicht in Berührung mit der Luft stehen lassen darf, weil sonst der Sauerstoff der Luft auf die Elektrode positive Ladung gäbe und sogar eine Einwirkung des oxydierten Saftes in der gleichen Richtung zu erwarten wäre.

Es konnte jedoch keine Versicherung dafür geben, dass der Presssaft während des Verfahrens durch den Sauerstoff gar nicht oxydiert wurde. Aber es scheint mir wahrscheinlich, dass dabei das Eindringen des Sauerstoffs grösstenteils zurückgehalten werden konnte, denn die schwarze Färbung des Presssaftes, die durch den geringen beigemengten Sauerstoff hervorgerufen wird, kam gar nicht in diesem Falle zum Vorschein.

Mehrmalige Versuche ergaben stets dieselben Resultate, wovon die Tabelle 2 und die Abb. 2 ein Beispiel geben.

Es lässt sich nun entnehmen, dass wir es hier mit der charakteristischen Potentialzeitkurve zu tun haben, d. h. das Potential von dem Anfangswert, $E_h = +350$ mV aus zuerst bis auf ein Potentialniveau, $E_h = +60$ mV,

TABELLE 2.

*Redoxpotential des Presssaftes von Vicia Faba. Bei 30°C.
unter der anaeroben Bedingung.*

Zeit in Min.	Eh in Millivolt	Zeit in Min.	Eh in Millivolt
0	+350	255	+10
15	+251	270	-4
30	+193	285	-38
45	+155	300	-65
60	+131	315	-96
90	+118	330	-133
105	+97	360	-182
120	+81	390	-234
135	+73	405	-246
150	+67	420	-260
165	+63	435	-261
180	+60	450	-264
195	+59	465	-266
210	+57	480	-267
225	+57	495	-267
240	+34	510	-267

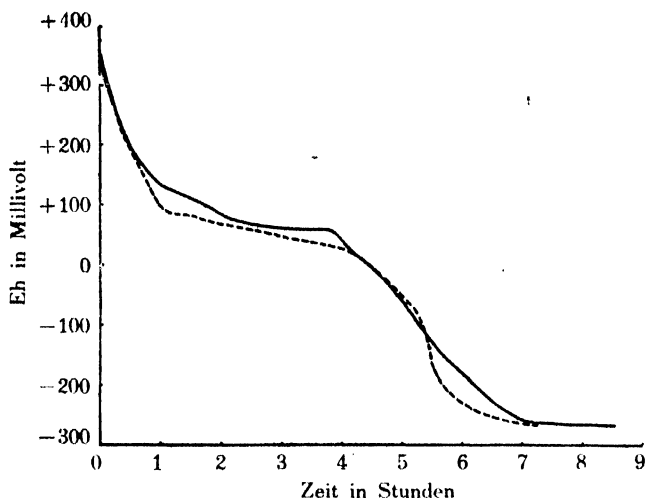


Abb. 2. Redoxpotentialzeitkurven der Presssäften von *Vicia Faba*. Bei 30°C, unter der anaeroben Bedingung.

----- junge Pflanzen, ————— erwachsene Pflanzen.

worauf es etwa 2½ Stunden lang liegen blieb, und dann wiederum bis auf den Endpotentialwert, $E_h = -267$ mV, worauf es beinahe konstant blieb, abfällt. Hier ergibt sich also die bemerkenswerte Sachlage, dass der betreffende Presssaft imstande ist, einerseits den Endpotentialwert von -267 mV herbeizuführen, andererseits den Potentialabfall um den Wert etwa $+60$ mV zu verzögern. Was nun die Wasserstoffionenkonzentration des Presssaftes anbelangt, so wurde pH nach etwa drei Stunden ungefähr um den Wert von 5,95 verhältnismässig lange Zeit, etwa 6 Stunden, gepuffert, wie im vorigen Abschnitt gezeigt wurde. Daraus geht hervor, dass das Redoxpotential, das in diesem Versuch erhalten wurde, lediglich durch das Redoxsystem im Presssaft, unabhängig von der pH-Veränderung, bedingt werden muss.

Bloss mit diesem Ergebnisse lässt sich jedoch noch nicht beurteilen, worin die Ursache dieser Verzögerung des Potentialabfalls zu suchen ist. Auf diese Erscheinung werden wir später zurückkommen.

3. Das Redoxpotential des Presssaftes der jungen Pflanze.

Da nun die Frage entsteht, inwieweit das Redoxpotential des Presssaftes durch das Alter der angewandten Pflanze beeinflusst wird, scheint es wünschenswert den Redoxpotentialverlauf des Presssaftes von den Keimpflanzen kennen zu lernen.

Der diesbezügliche Versuch wurde folgenderweise ausgeführt: Als Versuchsmaterial wurden Epikotylen der betreffenden Pflanze, die vier oder fünf Tage alt nach der Keimung und etwa zwei oder drei cm lang waren, benutzt. Die Versuchsanordnung war hier dieselbe wie beim vorigen Versuch.

Die Ergebnisse sind in Tabelle 3 zusammengestellt und in Abb. 2 graphisch wiedergegeben.

TABELLE 3.

*Redoxpotential des Presssaftes von den Keimpflanzen.
Bei 30°C, unter der anaeroben Bedingung.*

Zeit in Min.	Eh in Millivolt	Zeit in Min.	Eh in Millivolt
0	+ 339	255	+ 16
30	+ 191	285	— 28
45	+ 137	315	— 75
60	+ 92	315	—174
75	+ 84	360	—211
105	+ 70	375	—232
135	+ 63	405	—251
195	+ 41	435	—265
225	+ 34	450	—266

Aus diesen Ergebnissen geht hervor, dass sowohl der Potentialverlauf als auch der Endpotentialwert bei diesem Versuch fast ähnlich wie beim vorigen an der erwachsenen Pflanze waren.

Man darf aus diesem Versuch schliessen, dass das Redoxpotential des Gewebepresssaftes unabhängig von dem Alter der Pflanzen charakteristisch bestehen bleibt, mit anderen Worten, dass schon im Keimlinge ein und dasselbe Redoxsystem wie in ihrer späteren Lebenszeit in den betreffenden Geweben beteiligt ist.

4. Weitere Versuche über das Redoxpotential des Presssaftes von *Vicia Faba* in Gegenwart einiger Redoxindikatoren.

Der Zweck dieser Versuche ist zu prüfen, wie sich das Redoxsystem, welches in den vorigen Versuchen mit Hilfe der Elektrode im Presssaft festgestellt wurde, gegenüber einigen reversiblen Farbstoffsystemen verhält. Dabei ist es wünschenswert, nicht nur eine eintretende Reduktion des Farbstoffs zu beobachten, sondern dabei auch immer eine elektrometrische Bestimmung des Redoxpotentials vorzunehmen.

Die Versuchsanordnung war hier auch dieselbe wie bei den vorigen Versuchen, ausgenommen den Zusatz der Redoxindikatoren in den betref-

fenden Presssaft in dem Grade, dass die betreffende Farbe erst erkannt werden konnte. Als Redoxfarbstoffsysteme wurden Methylenblau, Indigotetrasulfonat, Indigotrisulfonat, Janusgrün und Neutralrot benutzt. Die Normalpotentialwerte dieser Farbstoffsysteme sind wie folgt:

Indikator	E_o' bei $pH=6,00$	Indikator	E_o' bei $pH=6,00$
Methylenblau	+ 47 mV	Janusgrün	- 183 mV
Indigotetrasulfonat.	+ 6 mV	Neutralrot	- 279 mV
Indigotrisulfonat.	- 28 mV		

Der Versuch zeigt, dass die ersteren vier Indikatoren: Methylenblau, Indigotetrasulfonat, Indigotrisulfonat und Janusgrün, durch den Presssaft völlig reduziert wurden, wie aus ihrer völligen Entfärbung beurteilt werden konnte. Diese Erscheinung ist überzeugend aus dem Grunde, dass ihre Normalpotentiale, alle höher als das Endpotential des Presssaftes sind. Aber bei Neutralrot, dessen Normalpotential niedriger als das Endpotential des Presssaftes ist, trat gar keine Entfärbung auf. Die Resultate dieser Versuche entsprechen durchaus den Erwartungen. Die bei diesen Fällen gefundenen Endpotentiale stimmten durchschnittlich mit dem ohne Zusatz von Redoxindikator festgestellten überein.

Als ein Beispiel von den dabei erhaltenen Resultaten ist der bei dem Zusatz von Janusgrün gefundene Potentialverlauf in Tabelle 4 wiedergegeben und in Abb. 3 graphisch dargestellt.

Die Farbveränderung von Janusgrün, welche durch seine Reduktion hervorgerufen wird, findet in zwei Stufen statt, und zwar erstens von blaugrün bis zu rot um $E_o' = +24$ mV bei $pH=5,8$ und zweitens von rot

TABELLE 4.

Redoxpotential des Presssaftes von Vicia Faba in Gegenwart von Janusgrün. Bei 30°C, unter der anaeroben Bedingung.

Zeit in Min.	Eh in Millivolt	sichtbare Beobachtung	Zeit in Min.	Eh in Millivolt	sichtbare Beobachtung
0	+369	— blaugrün	240	+ 10	— Entfärbung beginnt
15	+287		245	- 33	
30	+260		255	- 92	
45	+220		270	-126	
60	+167		285	-145	
90	+ 89	— etwas rötlich	300	-146	— völlig entfärbt
105	+ 80		315	-171	
120	+ 75		330	-195	
135	+ 66	— völlige Rotfärbung	345	-213	
165	+ 59		360	-228	
180	+ 57		375	-232	
195	+ 53		390	-232	
210	+ 50				

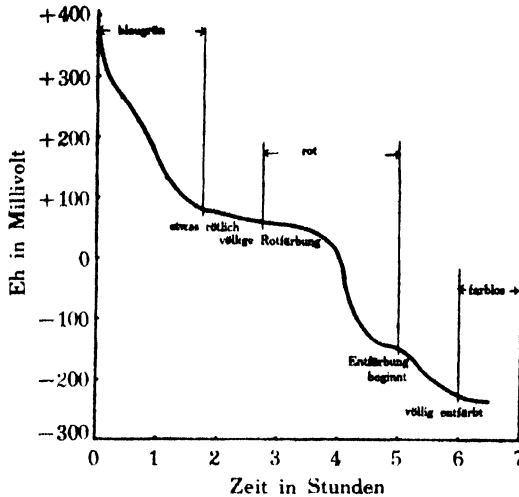


Abb. 3. Redoxpotential des Presssaftes von *Vicia Faba* in Gegenwart von Janusgrün. Bei 30°C, unter der anaeroben Bedingung.

bis zu farblos um $E'_0 = -183$ mV bei $\text{pH} = 6,0$. Das in den Presssaft hinzugefügte Janusgrün entfärbte jeder für sich Hand in Hand mit den entsprechenden Potentialbereichen im Verlauf des Potentialabfalls.

Alle diese Ergebnisse führen uns zu dem Schluss, dass das Endpotential des Presssaftes von *Vicia Faba* zwischen dem $E_h = -183$ mV, d. h. dem Normalpotential des Janusgrüns bei $\text{pH} = 6,00$, und dem $E_h = -279$ mV, d. h. dem Normalpotential des Neutralrotes bei $\text{pH} = 6,00$, liegt und dass der potentiometrisch gemessene Potentialwert im grossen und ganzen mit dem, welcher von dem Verlauf der Entfärbung der Redoxindikatoren gefolgert wird, im Einklang steht.

5 Der Einfluss des KCN-Zusatzes auf den Potentialverlauf des Presssaftes.

Ferner habe ich den Einfluss der Blausäure untersucht. Der Versuch wurde folgendermassen ausgeführt: In bezug auf die Konzentration des KCN wurden sowohl M/2000 und M/100 als auch M/10 benutzt. Zum Beispiel wurde 1 ccm der M/100 - Lösung zu 19 ccm des Presssaftes hinzugefügt, damit die Endkonzentration des KCN beträgt M/2000. Dieser mit KCN gemischte Presssaft wurde in das Elektrodengefäss gebracht. Methodisch ging ich sonst ebenso vor, wie in den vorhergehenden Abschnitten dargelegt wurde.

TABELLE 5.

Redoxpotentiale des Presssaftes von Vicia Faba bei dem Zusatz des KCN. Bei 30°C, unter anaeroben Bedingung.

M/2000-KCN		M/100-KCN		M/10-KCN	
Zeit in Min.	Eh in Millivolt	Zeit in Min.	Eh in Millivolt	Zeit in Min.	Eh in Millivolt
0	+431	0	+260	0	+121
30	+343	15	+160	5	+80
60	+245	30	+133	10	+59
90	+192	90	+53	15	+49
150	+159	120	+20	30	+22
180	+130	150	-19	60	+11
210	+102	180	-46	90	-31
240	+86	210	-76	120	-52
300	+69	240	-106	150	-90
330	+57	270	-137	180	-113
360	+21	360	-190	210	-133
390	-190	390	-211	240	-152
420	-233	420	-222	270	-163
450	-244	450	-229	300	-174
480	-249	480	-235	330	-184
510	-249	510	-238	360	-195
540	-256			390	-218
570	-255			420	-230
600	-256			450	-243
630	-256			480	-250
660	-256			510	-253

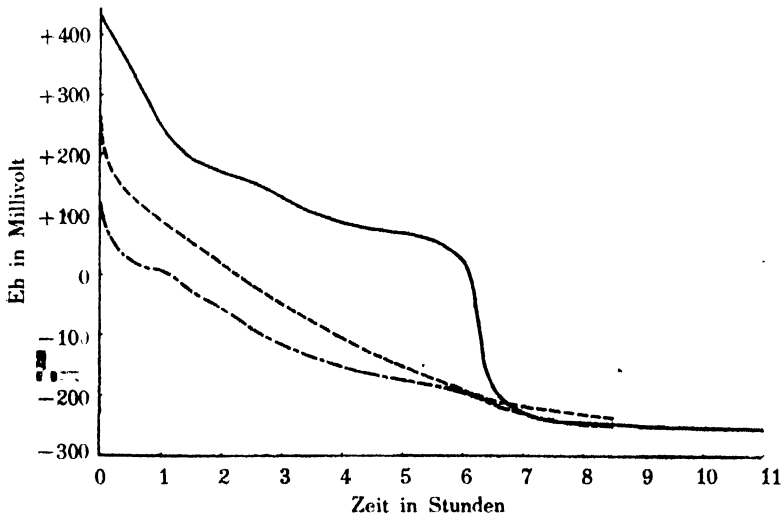


Abb. 4. Redoxpotentiale des Presssaftes von *Vicia Faba* bei dem Zusatz des KCN. Bei 30°C, unter der anaeroben Bedingung.

--- M/10-KCN-Zusatz, -.-.- M/100-KCN-Zusatz, — M/2000-KCN-Zusatz.

Die dabei erhaltenen Ergebnisse sind in Tabelle 5 zusammengestellt und in Abb. 4 graphisch wiedergegeben.

Wenn wir jetzt diese Ergebnisse überblicken, so ergibt sich folgendes: An erster Stelle ist augenfällig, dass Cyanid in M/2000 keinen nennenswerten Effekt auf den Potentialverlauf ausübt, während die Potentialverläufe bei M/10- und M/100-KCN ohne Verzögerungen der Potentialabfälle schnelle Drifte erleiden. Die Endpotentialwerte standen annähernd im Einklang mit dem ohne Zusatz des KCN.

ELEMA, KLUYVER und VAN DALFSEN ('34) berichteten, dass die Zugabe einer geringen Menge KCN zum Medium zur Änderung des Stoffwechsels von denitrifizierenden Bakterien führte, und daneben die Reduktionsniveaus im Medium verändert wurden. BECK und ROBIN ('34) teilten mit, dass Cyanid selbst in einer zur Hemmung der Atmung genügenden Konzentration keinen bemerkbaren Einfluss auf die aerobe Reduktionsintensität der Hefen ausübt. Nach KORR ('35) übt KCN keinen Einfluss auf das Redoxpotential der Leuchtbakteriensuspension aus.

So entsteht nun die Frage, warum KCN den Potentialverlauf ohne zeitliche Verzögerung des Potentialabfalls glatt und gleichmässig beeinflusst? Um diese Frage klarzustellen, tut es not, sich mit der weiteren Untersuchung noch eingehender zu beschäftigen.

6. Die Elektrotitration des Presssaftes von *Vicia Faba*.

Die in den vorigen Abschnitten mitgeteilten Ergebnisse zeigen eindeutig, dass dem Verlauf des Presssaftpotentials eine charakteristische Abfallsverzögerung eigen ist.

Da das im Presssaft sich befindende Redoxsystem nicht einzig, sondern zusammengesetzt ist, so lässt sich fragen, ob es von Bedeutung ist oder nicht, die Titrationskurve für den Presssaft festzustellen. Es scheint mir interessant zu sein zu prüfen, wie sich der Presssaft bei der Titration mit Hilfe der Kaliumferricyanidlösung im betreffenden Potentialbereich verhält. Diese Sachlage gab mir Anlass, die Titrationskurve für den Presssaft herzustellen.

Als Elektrodengefäß wurde der in Abb. 5 wiedergegebene etwa 25 ccm haltige Apparat benutzt, welcher mit einer Platinelektrode, einem KCl-Agarheber, der zwei Hineinsteckstützen, deren eine für eine Mikrobürette und eine andere für einen Rührer verwendet wurde, einem Sicherheitsröhrchen und einem Gaseinleitungsrohr ausgerüstet war. Zunächst wurde das Elektrodengefäß, worin 15 ccm von Presssaft, wie früher hergestellt, einpipettiert war, im Wasserthermostat (30°C) aufgestellt, und

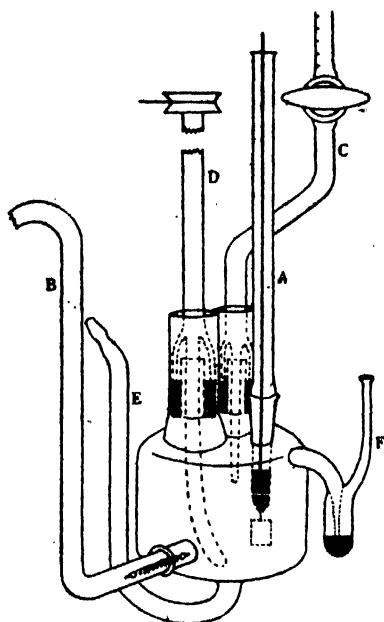


Abb. 5. Elektrotitrationsgefäß. A: Elektrode, B: KCl-Agarheber, C: Mikrobürette, D: Rührer, E: Gaseinleitungsrohr, F: Sicherheitsröhrchen.

sogleich das Wasserstoffgas durch das Gaseinleitungsrohr eingeleitet, um die im Gefäß befindliche Luft zu vertreiben, und zugleich den Presssaft völlig reduziert vorliegen zu lassen. Nach der Einstellung des Potentials, welche durch die Einleitung des Wasserstoffgases in kurzer Zeit erreicht werden konnte, wird das Stickstoffgas an Stelle des Wasserstoffgases eingeleitet. Dabei muss man dies so vorsichtig durchführen, dass dieses Gas von einer Bombe aus über geglühte Kupfernetze, welche vorher vollständig reduziert waren, übergeleitet und dann durch die alkalische Lösung des Natriumhydrosulfits geführt wurde. Nach etwa 30 Minuten wurde die Titration mit Hilfe der Mikrobürette ausgeführt, wobei der Rührer in Gang kam, während das Stickstoffgas fortgesetzt noch weiter eingeleitet wurde.

Als Oxydationsmittel wurde hierzu 0,01 M-Kaliumferricyanidlösung, die vorher im Phosphatpuffergemisch ($\text{pH}=5,91$) aufgelöst wurde, um die betreffende Lösung mit pH des Presssaftes übereinstimmen zu lassen, benutzt. Es war hier erforderlich, dass auf die Kaliumferricyanidlösung in der Mikrobürette eine geringe Menge, etwa 1 cm dick, Paraffinöl gesetzt wird, um den Presssaft vor dem Einfluss der atmosphärischen Luft zu schützen. Überdies muss man die Titration in möglichst kurzer Zeit, etwa 5 Minuten, abschliessen.

Das erhaltene Ergebnis ist in Tabelle 6 zusammengesetzt und in Abb. 6 graphisch wiedergegeben.

Es ist ersichtlich, dass sich die erhaltene Elektrotitrationskurve nicht typisch, sondern beinahe S-förmig erwies. Im diesen Versuch verändert sich die Farbe des Presssaftes im Laufe der Titration allmählich bis zu braunrot und schliesslich bis zu braunschwarz, wie es bei der Oxydation durch die Luft der Fall war.

Nun betrug E' für diese Elektrotitrationskurve, d. h. das Potential bei

TABELLE 6.

Elektrotitration des Presssaftes von Vicia Faba.
Bei 30°C, im Stickstoffgas, Oxydationsmittel:
0,01 M-Kaliumferricyanidlösung.

Ablezen der Bürette in ccm	Prozent der Oxydation	Eh in Millivolt
0	0	-299
0,25	9,1	-247
0,50	18,2	-102
0,75	27,3	-31
1,00	36,4	+17
1,25	45,5	+30
1,50	54,5	+54
1,75	63,6	+122
2,00	72,7	+171
2,25	81,8	+193
2,50	90,9	+292
2,75	100,0	+333

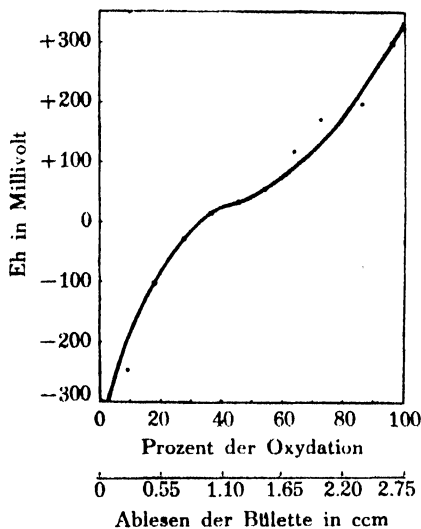


Abb. 6. Elektrotitrationskurve des Presssaftes von *Vicia Faba*.
 Bei 30°C, im Stickstoffgas, Oxydationsmittel: 0,01 M-Kalium-
 ferricyanidlösung.

dem halboxydierten Zustand des Presssaftes, etwa +45 mV bei pH=5,91. Es scheint mir wahrscheinlich, dass dieser Potentialwert sich nun mit dem Niveau, das sich bei der Verzögerung des Potentialabfalls in vorigen Versuchen erwies, reimt. Wenn dies zuträfe, so wäre man imstande zu sagen, dass sich ein gewisses Redoxsystem in diesem Potentialbereich beherrschend beteiligt.

7. *Beobachtungen der Potentialveränderungen von einigen Substraten unter der Einwirkung eines gewissen aus den Pflanzen extrahierten Enzyms.*

Es ist selbstverständlich, dass sich die Wasserstoffübertragung zwischen Substraten (Wasserstoffdonatoren) und Wasserstoffakzeptoren unter der Einwirkung der Dehydrogenase im Redoxpotential widerspiegeln muss. Diese Berücksichtigung zwingt mich zu erforschen, wie sich die Potentiale einiger Substrate durch ein gewisses Enzym verändern.

Zur Ausführung dieses Versuches dienten folgende Substanzen: Glukose, Globulin, Phenylalanin und Cystein. Die Extraktion der angewandten Enzyme gelang mir auf folgende Weise. Zum Beispiel wurden 10 gr von demgleichen Material, wie dem im Presssaftversuch angewandten, in einem Mörser zerrieben und dann 15 ccm des Glyzerins unter Umrührung hinzugefügt. Nachdem dieses antiseptisch behandelte Gemisch bei der Zimmertemperatur 24 Stunden lang mit der zeitlichen Umrührung stehen blieb, wurde es zentrifugiert. Die dabei erhaltene klare Oberflüssigkeit wurde in diesem Versuch als Enzymlösung verwandt. In folgenden Versuchen wurden jedesmal neu hergestellte Enzymlösungen angewandt.

Das Gemisch von der Substratlösung, die im Phosphatpuffergemisch ($\text{pH}=6,23$) gelöst war, mit dem Enzym wurde in das Elektrodengefäß eingebracht. Die übrige Versuchsanordnung war ganz gleich wie vorher.

(A) Glukose.

- (1) 15 ccm der 3%igen Glukoselösung mit 10 ccm des Enzyms.
- (2) 15 ccm der 3%igen Glukoselösung mit 10 ccm des gekochten Enzyms.
- (3) nur 3%ige Glukoselösung.

In Tabelle 7 und in Abb. 7 sind die erhaltenen Ergebnisse wiedergegeben.

Wie aus diesen Ergebnissen ersichtlich ist, fanden bei den letzteren Fällen gar keine nennenswerten Potentialveränderungen statt, während es sich beim ersteren Falle etwas anders verhielt, indem das Potential zunächst sehr langsam und nach etwa 20 Stunden plötzlich bis zum $+18 \text{ mV}$ zur Folge hatte.

(B) Globulin.

Die angewandte Globulinlösung wurde folgendermassen bereitet. Globulin wurde nach BERGMANN und LERVAS ('33) aus Saubohne *Vicia Faba*, hergestellt und nach der antiseptischen Behandlung im Eisschrank behalten. Nach der Zentrifugierung wurde es in dem NaCl-haltigen (5%) Phosphat-

TABELLE 7.

Potentialveränderungen der Glukoselösung unter Einwirkung des Enzyms, das aus Vicia Faba hergestellt wird. Bei 30°C, unter der anaeroben Bedingung, 3%ige Glukoselösung, pH=6,23.

3%ige Glukoselösung mit dem Enzym				3%ige Glukoselösung mit dem gekochten Enzym		3%ige Glukoselösung ohne Enzym	
Zeit in Std.	Eh in Millivolt	Zeit in Std.	Eh in Millivolt	Zeit in Std.	Eh in Millivolt	Zeit in Std.	Eh in Millivolt
0	+421	23, 25	+138	0	+423	0	+504
0, 5	+418	23, 5	+123	0, 5	+422	0, 5	+504
1, 0	+413	24, 0	+115	1, 5	+420	1, 0	+502
2, 0	+404	25, 0	+ 85	2, 5	+418	1, 5	+504
3, 0	+398	26, 0	+ 50	3, 5	+416	2, 0	+502
4, 0	+393	27, 0	+ 34	4, 5	+416	4, 0	+495
5, 0	+387	28, 0	+ 34	5, 5	+416	5, 0	+483
6, 0	+381	29, 0	+ 30	6, 5	+416	6, 0	+485
9, 0	+372	30, 0	+ 27	20, 5	+406	7, 0	+485
10, 0	+366	31, 0	+ 27	21, 5	+406	22 5	+475
11, 0	+360	35, 0	+ 24	22, 5	+405	24, 0	+469
12, 0	+357	36, 0	+ 23	23, 5	+404	26, 0	+469
13, 0	+354	46, 5	+ 20	24, 5	+403	28, 0	+469
14, 0	+350	47, 5	+ 19	25, 5	+403	30, 0	+468
15, 0	+343	48, 5	+ 19	26, 5	+402	47, 0	+449
16, 0	+338	49, 5	+ 18	27, 5	+401	48, 0	+445
18, 0	+320	53, 0	+ 18	28, 5	+400	51, 0	+445
20, 0	+297	54, 0	+ 18	29, 5	+400		
22, 0	+236			30, 5	+399		

puffergemisch (pH=6,23) gelöst, damit die Konzentration des zentri-fugierten Globulins 3% beträgt.

- (1) 15 ccm der obengenannten Globulinlösung mit 10 ccm des Enzyms.
- (2) 15 ccm der obengenannten Globulinlösung mit 10 ccm des gekochten Enzyms.

Die erhaltenen Ergebnisse sind in Tabelle 8 zusammengestellt und in Abb. 7 wiedergegeben.

Wie aus Tabelle 8 und Abb. 7 ersichtlich ist, fiel das Potential der Globulinlösung mit dem Enzym erst nach 25 Stunden stark ab, wie es bei dem Glukoseversuch der Fall war, während beim Versuch mit dem gekochten Enzym keine nennenswerte Potentialveränderung auftrat. Es sei hier bemerkt, dass der Endpotentialwert beim Enzymversuch etwa +31 mV betrug.

(C) Phenylalanin.

15 ccm der M/100-Phenylalaninlösung mit 10 ccm des Enzyms.

In Tabelle 9 und in Abb. 7 sind die Resultate angegeben.

TABELLE 8.

Potentialveränderungen der Globulinlösung unter Einwirkung des Enzyms, das aus Vicia Faba hergestellt wird. Bei 30°C, unter der anaeroben Bedingung, 3%ige Globulinlösung, pH=6,23.

3%ige Globulinlösung mit dem Enzym				3%ige Globulinlösung mit dem gekochten Enzym	
Zeit in Std.	Eh in Millivolt	Zeit in Std.	Eh in Millivolt	Zeit in Std.	Eh in Millivolt
0	+451	18,0	+345	0	+446
2,0	+430	18,5	+342	0,5	+439
2,5	+422	25,0	+301	1,0	+438
3,5	+422	25,5	+297	1,5	+437
4,5	+413	26,0	+284	2,5	+437
6,0	+411	26,5	+271	3,0	+437
6,5	+407	27,0	+262	3,5	+437
7,0	+402	27,5	+247	4,0	+435
7,5	+394	28,0	+226	5,0	+436
8,0	+393	28,5	+187	10,0	+431
8,5	+393	29,0	+133	10,5	+430
9,5	+389	29,5	+65	11,0	+428
10,5	+384	33,0	+52	21,0	+418
11,5	+383	33,5	+44	22,0	+418
12,0	+375	34,0	+40	23,0	+418
12,5	+373	34,5	+38	24,0	+418
13,0	+370	35,0	+38	25,0	+417
13,5	+367	35,5	+38	27,0	+416
14,5	+365	44,5	+35	29,0	+416
15,5	+361	47,5	+35	44,0	+402
16,5	+357	50,5	+31	46,0	+402
17,5	+346	52,5	+31		

TABELLE 9.

Potentialveränderung der Phenylalaninlösung unter Einwirkung des Enzyms, das aus Vicia Faba hergestellt wird. Bei 30°C, unter der anaeroben Bedingung, M/100-Phenylalaninlösung, pH=6,23.

Zeit in Std.	Eh in Millivolt	Zeit in Std.	Eh in Millivolt
0	+439	8,5	+290
0,5	+423	9,0	+258
1,0	+409	9,5	+188
2,0	+402	10,0	+119
2,5	+397	11,0	+77
3,0	+392	11,5	+53
3,5	+385	12,0	+38
4,5	+374	12,5	+30
5,0	+371	13,0	+30
5,5	+364	13,5	+30
6,0	+359	21,5	+27
6,5	+349	24,5	+28
7,0	+342	26,5	+30
7,5	+333	28,5	+30
8,0	+314		

Auch im diesen Falle ist ersichtlich, dass das Potential erst nach etwa 9 Stunden einem ziemlich plötzlichen Abfall unterliegt, und schliesslich das Niveau von etwa $Eh = +30$ mV erreicht, wonach es beinahe konstant bleibt.

TABELLE 10.

Potentialveränderung der Cysteinlösung unter Einwirkung des Enzyms, das aus *Vicia Faba* hergestellt wird.
Bei 30°C, unter der anaeroben Bedingung,
M/100-Cysteinlösung, pH=6,23.

Zeit in Std.	Eh in Millivolt	Zeit in Std.	Eh in Millivolt
0	+418	6,0	-146
0,5	+365	6,5	-168
1,0	+334	7,0	-180
1,5	+296	7,5	-184
2,0	+269	8,0	-189
2,5	+229	8,5	-194
3,0	+181	9,0	-194
3,5	+47	9,5	-195
4,0	-26	10,0	-196
4,5	-81	10,5	-197
5,5	-127	11,0	-197

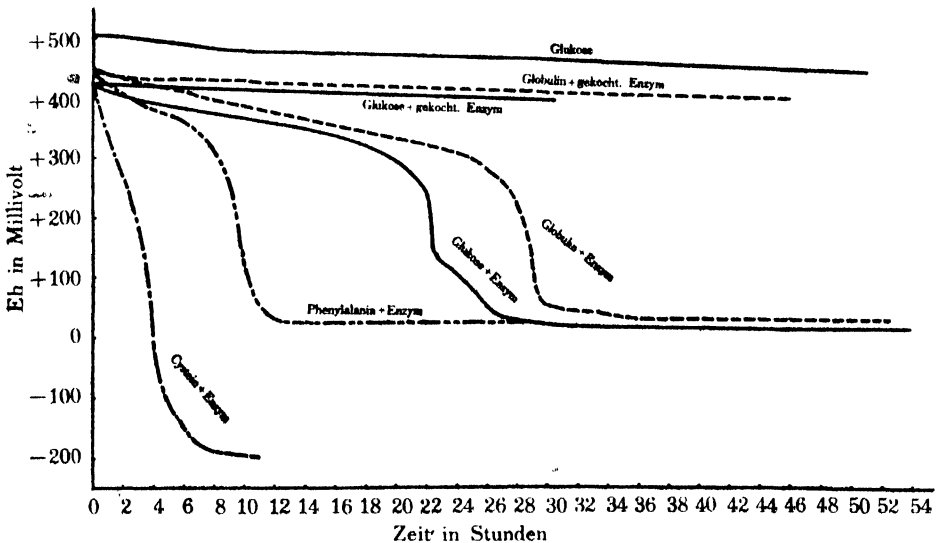


Abb 7. Potentialveränderungen der Substraten, Glukose, Globulin, Phenylalanin und Cystein, unter Einwirkung des Enzyms, das aus *Vicia Faba* hergestellt wird. Bei 30°C, unter der anaeroben Bedingung.

Beim Vergleich dieses Befundes mit denjenigen bei den vorigen Versuchen ist nun augenfällig, dass bei Phenylalanin trotz der schnellen Einstellung des Potentials der Endpotentialwert im allgemeinen mit dem der anderen Substrate in Übereinstimmung steht.

(D) Cystein.

15 ccm der $M/100$ -Cysteinlösung mit 10 ccm des Enzyms.

Die erhaltenen Ergebnisse sind in Tabelle 10 wiedergegeben und in Abb. 7 graphisch dargestellt.

Aus diesen Ergebnisse geht hervor, dass das Cystein imstande ist, einerseits unter der Einwirkung des Enzyms den Potentialwert von etwa $E_h = -197$ mV herbeizuführen, andererseits ihn in ausserordentlich kürzerer Zeit als bei den anderen Fällen zu erreichen.

DIXON und QUASTEL ('23) und DIXON und TUNNICLIFFE ('27) beobachteten, dass das Cystein, e. i. ein Sulfhydryls substanz, der Elektrode ein stark negatives Potential erteilen konnte. Von MICHAELIS und FLEXNER ('28) wurde festgestellt, dass die Cysteinlösung in Abwesenheit von Sauerstoff ein bestimmtes Potential an die indifferente Elektrode festsetzte, welches genügte, um die Erklärung der Reduktionen aller Redoxindikatoren durch Zellen unter der anaeroben Bedingung abzugeben. Aus diesen Tatsachen wäre es berechtigt anzunehmen, dass die hier gefundenen Ergebnisse sicherlich von Bedeutung vom zellphysiologischen Standpunkte aus sind.

Alle diese Ergebnisse führen uns ohne weiteres zum Schluss, dass die angewandten Substanzen, welche als Wasserstoffdonatoren angesehen zu werden scheinen, unter der Einwirkung des Enzyms, welches aus den angewandten Pflanzen hergestellt wird, die Dehydrierungserscheinungen herbeiführen können, sodass sie sich in den Potentialveränderungen an der Elektrode widerspiegeln können.

Man dürfte also hier Notiz von der Tatsache nehmen, dass die Endpotentialniveaus der Glukose-, Globulin- und Phenylalaninlösung dem beim Presssaftsversuch erhaltenen Niveau, wo der Potentialabfall beschwert wird, nahe liegen und das der Cysteinlösung im allgemeinen sich dem bei den betreffenden Versuchen erhaltenen Endpotentialniveau nähert.

8. *Die Methylenblaureduktion durch den Presssaft bei der Gegenwart einiger anderer Substrate.*

Es ist hier am Ort, einige Ergebnisse der Untersuchungen über die Dehydrogenase des Presssaftes einzuschalten. Wie es aus den vorhergehenden Versuchen ersichtlich war, wurde nachgewiesen, dass einige ange-

wandte Substrate unter der Einwirkung des Enzyms imstande waren, an den Elektroden Potentialveränderungen widerspiegeln zu lassen, was nichts anderes als das war, dass das betreffende Enzym eine Fähigkeit besass, auf solche Substrate dehydrierend zu wirken.

Diese Überlegung veranlasste mich den Methylenblaureduktionsversuch auszuführen. Der diesbezügliche Versuch wurde folgenderweise bewerkstelligt: Als Substrate wurden Glukose-, Phenylalanin-, Tyrosin- und Cysteinlösung angewandt. Diese Lösungen wurden in M/15-Phosphatpuffergemisch ($\text{pH}=6,23$) gelöst. Die Versuche wurden unter Anwendung des THUNBERG-Rohrs ausgeführt.

TABELLE 11.

Methylenblaureduktion durch den Presssaft bei Zugabe einiger Substrate. 0,5 ccm Presssaft, 1,0 ccm M/300-Tyrosinlösung oder M/100-Lösungen anderer Substrate, 0,1 ccm M/30000-Methylenblaulösung, als Kontrolle anstatt des Substrates Phosphatpuffer ($\text{pH}=6,23$) eingesetzt, bei 30°C .

Substrate	Entfärbungszeit in Min.	
Glukose	26	35
Phenylalanin	29	45
Tyrosin	21	36
Cystein	7	9
Kontrolle	32	52

Wie aus Tabelle 11 ersichtlich ist, wurde die Methylenblaureduktion bei der Zugabe dieser Substrate beeinträchtigt. Vor allem kam bei Cystein die merkbare Verkürzung der Entfärbung zustande. Diese Tatsache ist mit dem bei Cystein im vorigen Abschnitt erhaltenen Ergebnis im Einklang, d. h. es kam in beträchtlich kurzer Zeit an das niedrige Endpotential.

IV. DISKUSSION

In den letzten Jahren wurden eine Reihe Untersuchungen veröffentlicht, die sich auf das Redoxpotential der Pflanzenzellen beziehen. Nach den Bestimmungen von BROOKS ('26) betrug das rH des Zellsaftes in *Valonia ventricosa* einen Wert von 17,9-18,4. RAPKINE und WURMSER ('26) haben für rH in Zellen von *Spirogyra* einen Wert von 14,4-17,6 bestimmt.

In bezug auf das Redoxpotential der höheren Pflanzen werden hier einige Arbeiten kurz erwähnt. WURMSER und GÉLOSO ('30) berichteten, dass eine Zelle zwei Redoxpotentiale zeigte, wovon eines ein Normalpotential von $rH=14$ des im reversiblen Gleichgewicht befindlichen Systems und das andere ein Grenzpotential darstellte. WURMSER ('30) nahm an, dass der reduzierende Zucker, der unter der anaeroben Bedingung aktive Formen durch tautomere Umlagerung zu bilden imstande war und sich in manchen Hinsichten gleicherweise wie das intrazelluläre Redoxsystem verhielt, $rH=15$ erwies. Nach MAYER und PLANTEFOL ('28) zeigte sich der filtrierte Pflanzenpresssaft $19 < rH < 20$ ebenso wie es bei den Geweben unter der aeroben Bedingung der Fall war, während sie unter der anaeroben Bedingung Thionin und Methylenblau ($rH=14-16$), aber nicht Janusgrün ($rH=5$), reduzieren konnten. KRASSINSKY ('30) bestimmte die Redoxpotentiale der verschiedenen Reservestoffbehältern, und zwar Kartoffelknollen, Zwiebeln und Rüben. Nach ihm schwankte die Grösse von rH der Zellen dieser Pflanzen um 17 herum und veränderte sich beim Auskeimen der Reservestoffbehälter im Sinne einer Zunahme, was vermutlich mit einer Intensivierung der Oxydationsprozesse verbunden war. WARTENBERG, HEY und URHAN ('35) fanden, dass die gesunden Kartoffelknollen ein positiveres Redoxpotential als die kranken Knollen herbeiführten.

MARCKS FRANKE ('37) untersuchte die Physiologie der pflanzlichen Virose und bestimmte dabei positivere Potentialwerte von $rH=15-15,1$ bei den viruskranken Pflanzen (*Nicotiana*, *Beta* und *Phaseolus*) als bei den gesunden Pflanzen, $rH=13,7-14,2$. Die Endpotentialwerte der entsprechenden Pflanzen nahm jeder für sich im Sinne der gleichen Verhältnisse wie beim diesen Falle ab. Aber KAUSCHE ('37) berichtete, dass die Potentiale der viruskranken Pflanzen im allgemeinen im negativeren Gebiet der Potentialskala als die nicht beimpften lag. ROSS ('38) bestätigte, dass die Redoxpotentiale der Maispflanzen im allgemeinen zwischen $Eh = +125$ mV und $Eh = -5$ mV lagen und die Potentialdifferenz zwischen den grünen ($pH=5,25-5,76$) und den chlorotischen ($pH=5,30-5,89$) Pflanzen 20–70 mV betrug. Er kam zum Schluss, dass die erhöhte Atmung bei diesen Eisenmangelpflanzen gleichfalls das Sinken des Redoxpotentials mitbedingte.

Wie aus meinen vorigen Versuchen ersichtlich ist, beträgt das Redoxpotential des Presssaftes von etiolierten Pflanzen, *Vicia Faba*, im allgemeinen $Eh = +60$ mV bei $pH=5,95$, nämlich $rH=13,9$, im beschwerten Potentialbereich. Dank den obengenannten Arbeiten liegt uns als Tatsache

bewiesen vor, dass die Redoxpotentiale der Presssäfte der höheren Pflanzen wesentlich nicht so weit von dem hier gefundenen $rH=13,9$ abweichen. Aus dieser Tatsache wäre es berechtigt anzunehmen, dass man diesen Redoxpotentialwerten eine besonders wichtige Bedeutung zuschreiben müsste.

Die biologisch wichtigen Redoxsysteme sind von vielen Forschern untersucht worden (man vgl. hierfür WURMSER und FLITTI-WURMSER '37 und '38, und BARRON '39). In bezug auf den Mechanismus des Redoxpotentials der Zellen ist bisher folgendes ausgemacht worden: Von MACHLIS und GREEN ('33) wurde bewiesen, dass das sogenannte aerobe Potential der Zellen durch das kinetische Gleichgewicht bestimmt wird und keine thermodynamische Bedeutung besitzt. Mit anderen Worten es ist hervorzuheben, dass das aerobe Potential, soweit es mittels der Redoxindikatoren bestimmt wird, die Resultante nicht eines statischen Gleichgewichts, sondern eines dynamischen ist, was auch von MICHAELIS ('33) und BECK und ROBIN ('34) vertreten wurde. Die dynamische Eigentümlichkeit des aeroben Redoxpotentials ist schon von MICHAELIS und FLEXNER ('28) und WURMSER ('30) zugegeben worden.

Nun ist dieser Sachverhalt von GREEN, STICKLAND und TARR ('34) auf das anaerobe Redoxpotential erweitert worden. Nach ihnen wird auch das anaerobe Redoxpotential durch den kinetischen Faktor in demselben Sinne bedingt, wie es bei dem aeroben Redoxpotential der Fall ist, d. h. durch das relative Geschwindigkeitsverhältnis zwischen den zwei sich entgegenlaufenden Prozessen, in deren einem der Indikator durch das negativere Dehydrogenase-System reduziert wird und in deren anderem auch gleichzeitig derselbe durch das positivere Dehydrogenase-System oxydiert wird.

Dies wurde auch von KORR ('35) bestätigt und ausserdem wurde folgendes angegeben, dass das Prinzip, welches durch die Indikatormessung herbeigeführt war, zweifellos auch für das potentiometrisch gemessene Potential gelten konnte, denn sowohl der Indikator als auch die Elektrode funktionierten gleichermassen als die Elektronenakzeptoren (CLARK '25 und DIXON '27).

Es wäre aber verfrüht, den Mechanismus des Redoxpotentials in den Zellen der höheren Pflanzen einzig und allein auf Grund von den an ihren Presssäften gefundenen Ergebnissen erschöpfend zu diskutieren. Nach dieser Richtung liegen in der Tat noch weitere und eingehende Untersuchungen vor uns.

Was nun die Verzögerung des Potentialabfalls betrifft, so kommt es

mir vor, dass sofern ein gewisses Redoxsystem im betreffenden Potentialbereich über ein negativeres irgendwie überwiegt, kein Potentialwiederabfall erfolgen kann, sodass die dabei auftretende Verzögerung des Potentialabfalls der beschwerenden Wirkung des ersteren Redoxsystems zugeschrieben werden muss. Wenn dies zuträfe, wäre man imstande zu sagen, dass die Blausäure das erstere Redoxsystem im Sinne der Hemmung seiner beschwerenden Wirkung beeinflusste. Diese Erklärung hat wahrscheinlich mit der Meinung von GREEN, STICKLAND und TARR ('34) etwas Analoges gemeinsam, denn die Blausäure hält bekanntlich den Oxydationsprozess durchaus zurück. Es sei nun bemerkt, dass nach der Meinung von MARCKS FRANKE ('37) die bei den viruskranken Pflanzen auftretende Verlagerung des Horizontalwendepunktes auf die Bildung eines unter normalen Umständen nicht vorhandenen oder nicht wirksamen Redoxsystems zurückgeführt wird, dessen Beschwerung den geringen Potentialabfall im kritischen Bereich verursacht, während sich bei den gesunden der Potentialverlauf ähnlich verhält, wie es bei meinem Versuch der Fall ist. Darüber schrieb KAUSCHE ('37) folgendes: Als Kriterium für die mögliche Beschwerung des Redoxsystems durch einen Virusinfekt halten wir an den Unterschieden des konstanten Endpotentials fest. Die Grösse der Potentialverlagerung, also die Schnelligkeit und Art der Einstellung auf das Endpotential, hängt von vielen, zum Teil noch nicht erfassbaren Faktoren ab.

Betreffs des Endpotentials betrug es $E_h = -267$ mV bei $pH = 5,95$, bzw. $rH = 3,0$ im meinen Versuch. Nach MARCKS FRANKE ('37) betrugen die Endpotentiale der gesunden Pflanzen $rH = 5,3$ für *Nicotiana*, $rH = 2,8$ für *Beta* und $rH = 8,0$ für *Phaseolus*. Also ist das im meinen Versuch gefundene Endpotential etwas niedrig. Aber ist es jetzt schwer zu sagen, durch welches System dieser Wert verursacht wird. Im Zusammenhang mit diesem Sachverhalt sei nur bemerkt, dass die im Enzymversuch erhaltenen Ergebnisse, wo die Potentialveränderungen der Glukose-, Globulin-, Phenylalanin- und Cysteinlösung unter der Einwirkung des Enzyms bestimmt wurden, besonders meine Aufmerksamkeit anziehen.

V. ZUSAMMENFASSUNG

1. Die Wasserstoffionenkonzentration des Presssaftes der etiolierten Pflanze, *Vicia Faba*, wird um etwa $pH = 5,95$ ziemlich lange Zeit, etwa 9 Stunden, gepuffert, abgesehen von der späteren Abnahme.
2. Der Presssaft der betreffenden Pflanze erweist sich, eine Fähig-

keit zum charakteristischen Redoxpotentialverlauf zu haben. Er ist imstande, einerseits in der Potentialzeitkurve eine Verzögerung des Potentialabfalls um etwa $E_h = +60$ mV zu verursachen, andererseits den Endpotentialwert von -267 mV beim betreffenden pH einzustellen.

3. Die Redoxpotentialzeitkurve verläuft gleichartig, unabhängig von dem Alter der Pflanzen.

4. Der Presssaft reduziert Methylenblau, Indigotetrasulfonat, Indigotrisulfonat und Janusgrün, aber nicht Neutralrot.

5. Kaliumcyanid in der höheren Konzentration wirkt auf den Potentialverlauf des Presssaftes im Sinne der Hemmung der Abfallsverzögerung, aber nicht auf den Endpotentialwert.

6. Die Elektrotitrationskurve des Presssaftes gegenüber die Kaliumferricyanidlösung ist dargestellt.

7. Die Potentialveränderungen einiger Substanzen sind unter der Einwirkung des Enzyms, das aus der betreffenden Pflanze hergestellt wird, untersucht worden. Dabei betragen die Endpotentiale bei $pH = 6,23$ $E_h = +18$ mV für Glukoselösung, $E_h = +31$ mV für Globulinlösung, $E_h = +30$ für Phenylalaninlösung und $E_h = -197$ mV für Cysteinlösung.

8. Der Presssaft beeinträchtigt die Methylenblaureduktion bei der Gegenwart einiger Substrate: Glukose, Phenylalanin, Tyrosin und Cystein. Vor allem kommt bei Cystein eine starke Verkürzung der Entfärbungszeit zustande.

An dieser Stelle sei es mir erlaubt, Herrn Prof. Dr. Y. YAMAGUTI meinen herzlichsten Dank für die Anregung und Belehrung bei dieser Arbeit auszusprechen. Auch danke ich Herrn ausserordentlichem Prof. Dr. T. KOIZUMI, der mir in liebenswürdiger Weise manche Erleichterungen gewährte.

LITERATURVERZEICHNIS

- AUBEL, E., E. AUBERTIN und L. GENEVOIS, (1926). Sur le potentiel d'oxydo-réduction de la levure, des anaérobies facultatifs, des anaérobies stricts, et des milieux où vivent ces organismes. *Ann. Physiol. et Physicochim. Biol.*, **5**, 1.
- BARRON, E. S. GUZMAN, (1939). Cellular oxidation systems. *Physiol. Rev.*, **19** (2), 184.
- BECK, L. V. und J. P. ROBIN (1934). Effects of agents on the reduction of indicators and of cytochrome by yeast cells. *J. Cell. and Comp. Physiol.*, **4**, 527.
- BERGMANN, M. und L. LERVAS, (1933). Eiweissstoffe. G. KLEIN, Handbuch der Pflanzenanalyse. *Vierter Band, spezielle Analyse III.* Julius Springer, Wien, 344.
- BOYLAND, E. (1930). Phosphoric esters in alcoholic fermentation. III. The lag between phosphate esterification and carbon dioxide evolution. IV. Oxidation-reduction potentials of yeast preparations. *Biochem. J.*, **24**, 703.

- BROOKS, M. M. (1926). Studies on the permeability of living cells. VI. The penetration of certain oxidation-reduction indicators as influenced by pH; estimation of the rH of *Valonia*. *Am. J. Physiol.*, **76**, 360.
- , (1930). The pH and the rH of the sap of *Valonia* and the rH of its protoplasm. *Protoplasma*, **10**, 505.
- CANNAN, R. K., B. COHEN und W. M. CLARK, (1926). Studies on oxidation-reduction. X. Reduction potentials in cell suspensions. *Suppl.* **55**, to *Pub. Health Rep.*
- CLARK, W. M. (1925). Recent studies on reversible oxidation-reduction in organic systems. *Chem. Rev.*, **2**, 127.
- DIXON, M. (1927). On the mechanism of oxidation-reduction potential. *Proc. Roy. Soc., B*, **101**, 57.
- , und J. H. QUASTEL, (1923). A new type of reduction-oxidation system. Part I. cysteine and glutathione. *J. Chem. Soc.*, **123**, 2943.
- , und H. E. TUNNICLIFFE, (1927). On the reducing power of glutathione and cysteine. *Biochem. J.*, **21**, 844.
- ELEMA, B., A. J. KLUYVER und J. W. VAN DALFSEN. (1934). Über die Beziehungen zwischen den Stoffwechselvorgängen der Mikroorganismen und dem Oxydoreduktionspotential im Medium. I. Mitt. Versuche mit denitrifizierenden Bakterien. *Biochem. Z.*, **270**, 317.
- FROMAGEOT, CL. und P. DESNUELLE, (1935). Über das Oxydoreduktionspotential von Hefesuspensionen und einige damit im Zusammenhang stehende Fragen. *Biochem. Z.*, **279**, 34.
- , und G. BOST, (1937). Sur le pouvoir réducteur des levures vivantes au cours de la fermentation alcoolique. *Enzymologia*, **2**, 73.
- GAUTHERET, R. (1939). Potentiel d'oxydo-réduction des milieux de culture additionnés de colorants vitaux en présence de levures. *C. r. Acad. Sci. Paris*, **208**, 1840.
- GREEN, D. E., L. H. STICKLAND und H. L. A. TARR, (1934). Studies on reversible dehydrogenase systems. III. Carrier-linked reactions between isolated dehydrogenases. *Biochem. J.*, **28**, 1812.
- HEWITT, L. F. (1930). Oxidation-reduction potentials of cultures of haemolytic streptococci. I. *Biochem. J.*, **24**, 512.
- , (1930). Oxidation-reduction potentials of cultures of *C. diphtheriae* Ebenda, **24**, 669.
- , (1930). Oxidation-reduction potentials of staphylococcal cultures. I. Ebenda, **24**, 676.
- KAKUKAWA, T. (1938). Über das Redoxpotential der Suspension lebender Hefezellen. *Sci. Rep. Tōhoku Imp. Univ., Biol.*, **12**, 551.
- KAUSCHE, G. A. (1937). Zur Frage der Beziehungen zwischen Virusinfekt und Stoffwechselphysiologie bei pflanzlichen Viroten. *Biochem. Z.*, **294**, 365.
- KLUYVER, A. J. und J. C. HOGERHEIDE. (1934). Über die Beziehungen zwischen den Stoffwechselvorgängen der Mikroorganismen und dem Oxydoreduktionspotential im Medium. II. Mitt. Versuche mit Hefearten. *Biochem. Z.*, **272**, 197.
- und —, (1936). Beziehungen zwischen den Stoffwechselvorgängen von Hefen und Milchsäurebakterien und dem Redox-Potential im Medium. III. Neue Versuche mit Hefe-Arten. IV. Versuche mit Milchsäurebakterien. *Enzymologia*, **1**, 1.
- KNIGHT, B. C. J. G. (1930). Oxidation-reduction studies in relation to bacterial growth. I. The oxidation-reduction potential of sterile meat broth. *Biochem. J.*, **24**, 1066.
- , (1930). II. A method of poisoning the oxidation-reduction potential of bacteriological culture media. Ebenda, **24**, 1075.
- KORR, I. M. (1935). An electrometric study of the reducing intensity of luminous bacteria

- in the presence of agents affecting oxidation. J. Cell. and Comp. Physiol., **6**, 181.
- KRASSINSKY, N. (1936). Über Oxydations-Reduktions-Potentiale der Zellen der höheren Pflanzen. Protoplasma, **25**, 41.
- KUSNETZOW, S. I. (1931). The dependence of the oxidizing fermentations from the oxidation reduction potential of the external medium. Zentralbl. f. Bakteriologie, II. Abt., **83**, 37.
- LEHMANN, J. (1934). Zur Kenntnis biologischer Oxydations-Reduktionspotentiale. Vorläufige Potentialmessungen im System: Alkohol-Dehydrogenase-Acetaldehyd. Biochem. Z., **274**, 321.
- LIPMANN, F. (1934). Über die Hemmung der Mazerationssaftgärung durch Sauerstoff in Gegenwart positiver Oxydoreduktionssysteme. Ebenda, **268**, 205.
- , (1934). Versuche zur potentiometrischen Erfassung der Oxydoreduktionsvorgänge in gärendem Hefeextrakt. Ebenda, **274**, 329.
- , (1934). Potentiometrische Versuche über die Bildung des Gärungsreduktans. Ebenda, **274**, 412.
- MACHLIS, S. und D. E. GREEN, (1933). Intracellular oxidation-reduction studies. VII. Mechanism of reduction potentials in starfish sperm. J. Cell. and Comp. Physiol., **4**, 61.
- MARCKS FRANKE, H. (1937). Untersuchungen über die Physiologie der pflanzlichen Virose. Biochem. Z., **293**, 39.
- MAYER, A. und L. PLANTEFOL, (1928). Le pouvoir hydrogénant des tissus des végétaux et de leur constituants solubles. Ann. Physiol. et Physiochim. Biol., **4**, 297.
- MICHAELIS, L. (1933). Oxydations-Reduktions-Potentiale. Zweiter Auflage. Julius Springer, Berlin.
- und L. B. FLEXNER, (1928). Oxidation-reduction systems of biological significance. I. The reduction potential of cysteine: its measurement and significance. J. Biol. Chem., **79**, 689.
- und C. V. SMYTHE, (1936). Influence of certain dyestuffs on fermentation and respiration of yeast extract. Ebenda, **113**, 717.
- QUASTEL, J. H., M. STEPHENSON und M. D. WHETHAM, (1925). Some reactions of resting bacteria in relation to anaerobic growth, Biochem. J., **19**, 304.
- RAPKINE, L. und R. WURMSER, (1926). Sur le potentiel de réduction des cellules. C. r. S. c. Biol. Paris, **95**, 604.
- ROSS, H. (1938). Sulfat-, Nitratreduktion und Redoxpotential bei Eisenmangel in höheren Pflanzen. Flugschrift aus dem pflanzenphysiol. Inst. der Univ. Berlin.
- TANG, P. S. und C. Y. LIN, (1936). Studies on the kinetics of cell respiration. IV. Oxidation reduction potentials of *Chlorella* suspensions in light and in darkness. J. Cell. and Comp. Physiol., **9**, 149.
- WARTENBERG, H., A. HEY und O. URHAN, (1935). Die elektrometrische Pflanzgutwertbestimmung der Kartoffelknolle. I. Mitt. Arb. biol. Reichsanst. Land- u. Forstw., **21**, 331.
- , (1935). Die Platin-Gold-Differenz bei der Potentialbildung indifferenten Elektroden im Gewebeprei der Kartoffelknolle. Planta, **24**, 711.
- und A. HEY, (1936). Das Redoxpotential des Gewebepreises der Kartoffelknolle. III. Mitt. Ebenda **25**, 258.
- WURMSER, R. (1930). Oxydations et Réductions. Les Presses Universitaires de France, Paris.
- und J. GÉLOSO, (1930). Sur les potentiels d'oxydo-réduction intracellulaires. C. r. Soc.

Biol. Paris, 104, 135.

- und S. FILIPPI-WURMSER, (1937). Le rôle de la protéoflavine dans l'équilibre électrochimique des cellules. *Enzymologia*, 4, 137.
- und —, (1938). Le potentiel d'oxydoréduction du système d(-)alanine \rightleftharpoons pyruvate d'ammonium. *C. r. Soc. Biol. Paris*, 128, 133.
- und —, (1938). Le potentiel d'oxydoréduction du système cytochrome C \rightleftharpoons oxy-cytochrome C. *J. Chim. physique*, 35, 81.

REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY

36. OPHIUROIDEA OF THE MUTSU BAY AND VICINITIES¹⁾

By

HIKOSHICHIRO MATSUMOTO

(With Plates XXI-XXIII and 10 text-figures)

INTRODUCTION

The present material was collected by Professor HATAI, Professor HÔZAWA and their coöperators and assistants. It has been submitted by Professor HÔZAWA for study to the present writer, who distinguished the following forms in it.

Order Gnathophiurida

Family Amphiuridae

1. *Ophiopholis mirabilis*
2. *Ophiophragmus japonicus*
3. *Ophiophragmus japonicus* var. *parvus*, n. var.
4. *Amphipholis tetracantha*, n. sp.
5. *Amphipholis kochii*
6. *Amphiura sinicola*, n. sp.

Family Ophiotrichidae

7. *Ophiotrix marenzelleri*

Order Chilophiurida

Family Ophiolepididae

8. *Ophiura kinbergi*
9. *Ophiura sarsii*

Among them, the eighth species is Indo-Pacific in distribution, the ninth is circumpolar and the rest are Honshû species. One species, which is not represented in this collection, can be brought here into consideration: it is *Amphipholis pugetana*, which is known in Japan to range from the Okhotsk Sea to the vicinity of Kinkwasan, though ranges from Alaska as far southward as to Peru along the western coast of the New World. By far the most abundant of this collection is the first species, which ranges from the Okhotsk Sea to around Honshû, and the next abundant

¹⁾ Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 173

is the ninth, which is distributed widely over the North Pacific, the Arctic Ocean and the North Atlantic.

DESCRIPTION OF SPECIES

Ophiopholis mirabilis (DUCAN)

(Pls. XXI-XXIII, text-fig. 1)

CLARK, Mem. Mus. Comp. Zool., XXV, No. 1, 1915, p. 268;

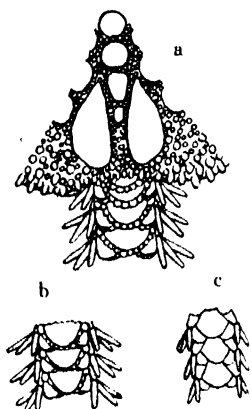
MATSUMOTO, Journ. Sci. Coll., Tokyo, XXXVIII, Art. 2, 1917, p. 160;

MATSUMOTO, Annot. Zool. Jap., IX, Pt. 4, 1918, p. 478.

Station 1; off Yunoshima; numerous specimens. Station 21; off Hanakuri; abundant specimens. Station 24; off Moura; numerous specimens. Station 26; off Futagojima; abundant specimens. Station 27; off the Biological Station; numerous specimens. Station 28; off the Biological Station; numerous specimens. Station 30; off Itazaki; abundant specimens. Station 41; off Okunai-mura; abundant specimens. Station 42; off Yomogida-mura; abundant specimens. Station 44; off Futatsuya-mura; abundant specimens. Station 61; off Bentenjima and Ōshima; numerous specimens. Station 62; off Ozawa; numerous specimens. Station 63; off Shukunobe; seven specimens. Station 68; off Jōgasawa; one specimen. Station 72; off Asadokoro; one specimen. Station 73; off Shimizugawa; numerous specimens. Station 74; off Karibazawa; three specimens. Station 76; off Myōmae; abundant specimens. Station 77; off Arito; numerous specimens. Station 80; off Ushinosawa; numerous specimens. Station 81; off Yokohama; numerous specimens. Station 82; off Hinoki; numerous specimens. Off and west of Ōshima; abundant specimens. Ōse, off Yunoshima; three specimens.

This well-known species is characterised by the presence of a pair of especially large supplementary dorsal arm plates, besides series of smaller supplementary ones, to each arm joint.

As observed in young specimens or on distal arm joints of adults, the paired large supplementary plates are ontogenetically early to appear, appearing decidedly earlier than the smaller supplementary ones. In life, this species is one of the most charmingly coloured ophiurans, being variously variegated, annulated and spotted with various combinations of various shades of yellow, green, brown, orange, red, purple, gray, white, etc. Some of the life studies by late artist Mr. SAKUMA on this problem are shown on the accompanying plates. The elaborate coloration of this



Text-fig. 1. *Ophiopholis mirabilis*.
×6. a. From above. b. Dorsal view
of three arm joints of middle part of
arm. c. Ditto of distal part of arm.

kind is often to meet with in those ophiurans, which climb on or live among weeds and zoophytes.

Ophiophragmus japonicus MATSUMOTO

(Text-fig. 2)

CLARK, loc. cit., 1915, p. 239;

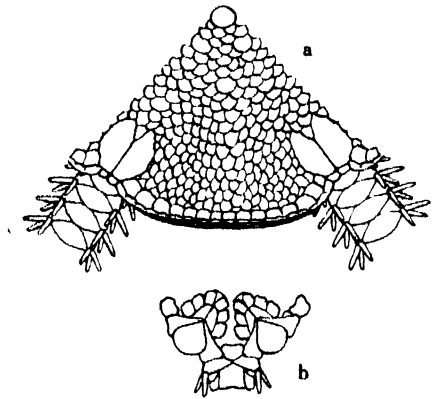
MATSUMOTO, loc. cit., 1917, p. 183;

MATSUMOTO, loc. cit., 1918, p. 478.

Amphiplus japonicus CLARK, Bull. Mus. Comp. Zool., LXII, No. 1918, p. 281.

Station 53; off Aburagawa; three specimens. Station 62; off Ozawa; one specimen. Off Gôzawa; five specimens.

Most of these specimens are young, only two from Station 53 being full-grown. All the specimens shows the characteristic frame-work of solid disk, having the special row of squarish marginal scales and a sort of fence of up-turned scales immediately outside them. In some specimens, the adoral shields meet with each other just inside the oral shield, while in others, they do not meet at all. The oral papillae are usually four on either side of the oral angle, but are sometimes five and sometimes three. When they are four or five in number, the outermost one arises from the oral plate and adoral shield or from the adoral shield and first ventral arm plate. When they are three, the oral structure is of course just the same as that of typical *Amphiodia*. There is no additional papilla just outside and above the infradental one, quite unlike in *Amphiplus*, the latter oral papilla being hence the highest of all the papillae in position. The adradial surfaces of the oral angles are rather even and nearly vertical, instead of being hollowed out obliquely downwards:—a feature common with *Amphipholis* but not with *Amphiplus*. In the writers opinion, the present species, as well as genus, belongs actually to the *Amphiodia*-group, but has nothing to do with the *Amphiplus*-group. As to the extent of the genus *Ophiophragmus*, the writer accepts LYMAN's original diagnosis, which



Text-fig. 2. *Ophiophragmus japonicus*.
×8. a. From above. b. Ventral view of two oral angles.

lays stress on the solid disk with the frame-work around it, though CLARK has tried to emend the genus, notwithstanding he was aware of a serious example of *Ophiophragmus chilensis* (MÜLLER & TROSCHEL) against his statement. No doubt, the present species is closely allied with *Ophiophragmus periercta* (CLARK) on the opposite coast of the North Pacific, though CLARK wants to refer the former to *Amphioplus* and the latter to *Amphiodia*. *Ophiophragmus*, as conceived by LYMAN, as well as by the writer, is valid and homogeneous, being intertropical in distribution, though a certain specific group of it might have arisen in, and be native to, the tropical and subtropical waters of the New World.

***Ophiophragmus japonicus* var. *parvus*, var. nov.**

(Text-figs. 3 and 4)

Ōse, off Yunoshima; three specimens.

Diameter of disk 6 mm. Length of arms 25 mm. or more. Width of arms at base 1 mm.

Disk subpentagonal, covered with fine, imbricating scales of irregular size, solid, with a frame-work of a row of turned-up scales around, though any row of large and squarish marginal scales immediately inside it is quite indistinct: — a distinctive feature in contrast to the typical form of the species. The primary plates are indistinct at least in adult, though may be distinct in young individuals. Radial shields pear-seed shaped, one third as long as the disk radius, twice as long as wide, joined in pairs along the outer two thirds the length, rather acutely pointed within. The squamation of the interbranchial ventral surfaces is finer than that of the dorsal side. Genital slits long.

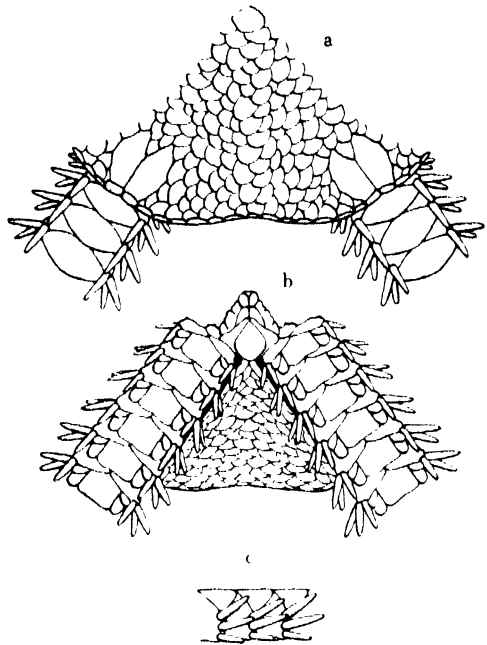
Oral shields rhomboidal, with the inner sides longer than the outer, with rounded angles, longer than wide. Adoral shields triangular, tapering inwards, meeting with each other just inside the oral shields. Four oral papillae on either side of the oral angle, close-set, forming a continuous row, blunt, none of them being operculiform, though the second outer one is the broadest; the infradental one is thick, while the other three are flattened and show a tendency of being imperfectly divided from one another.

Dorsal arm plates large, oval, very wide, with convex inner and outer borders, twice to two and a half times as wide as long, a little in contact with one another. Lateral arm plates low, inserted like as many wedges between both the successive dorsal and ventral arm plates above and

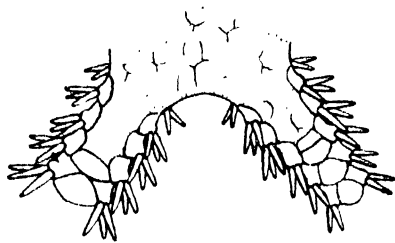
below, separated from each other above, more or less separated or just in contact below. First ventral arm plate very small. quadrangular, much wider than long. Those beyond pentagonal, with very large inner angle and linear or very slightly notched outer border, slightly wider than long, usually not in contact with one another. Three arm spines, conical. rather acute, subequal, though the upper two are more or less longer than the lowest, which is about as long as the corresponding arm joint. Two tentacle scales large, very flat, thin, the abradial one is the smaller of the two and overlaps the base of the adradial one, which is especially large and tongue-shaped.

Colour in alcohol, light yellow.

The present variety differs from the typical form of the species in the indistinctness of any row of large and squarish marginal scales, in the radial shields joined in pairs to a less extent and in the ventral arm plates being usually not in contact with one another. It appears probable, that the present variety, as well as species, is allied with the form imperfectly known under the name *Amphioplus megapomus* CLARK, though parts of its original description, which run as "upper arm plates tetragonal, broadly in contact," "apical ones of oral papillae widely separated from each other," "under arm



Text-fig. 3. *Ophiophragmus japonicus* var. *parvus*. $\times 10$. a. From above. b. From below. c. Lateral view of three arm joints near disk.



Text-fig. 4. *Ophiophragmus japonicus* var. *parvus*. $\times 12$. Specimen on way of regeneration of disk and some arms. Viewed from above.

plates much wider than long", &c., would not suit well for the present variety, as well as species.

One specimen, which must be eliminated from being the type of the variety, is interesting enough, exhibiting its being on the way of regeneration of disk and arms obviously after the loss of the original ones. The extent of the lost disk is indicated by the absence of the normal dorsal arm plates in a few basal arm joints. The disk covering acquired anew is a skin, in which calcification of plates and scales occurs.

***Amphipholis tetracantha*, sp. nov.**

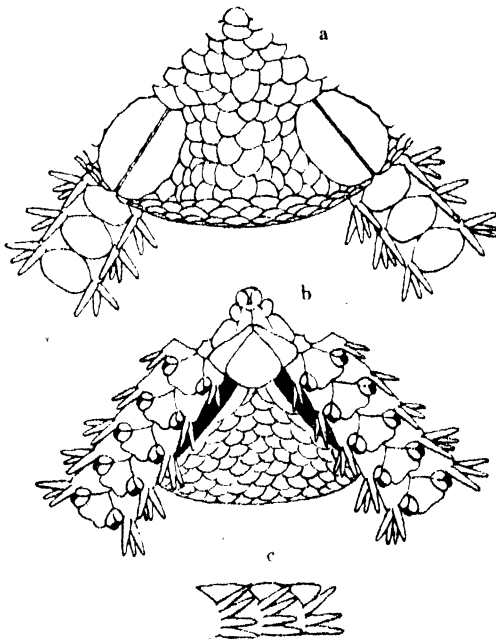
(Text-fig. 5)

Station 114; Ōma; one specimen.

Diameter of disk 3 mm. Length of arms 9 mm. Width of arms at base 0.5 mm.

Disk circular, covered with fine, thin, imbricating scales. Primary plates indistinct. Radial shields rather large, pear-seed shaped, slightly shorter than half the disk radius, about twice as long as wide, blunt both within and without, completely joined in pairs. Interbrachial ventral surfaces covered also with fine, thin, imbricating scales, which are finer than those of the dorsal side. Genital slits long.

Oral shields rhomboidal, with very acute inner and rounded lateral and outer angles, with inner sides longer than the outer, about as wide as long. Adoral shields triangular, long and narrow, wider outwards than inwards; meeting with each other within. Three oral papillae on either side of the oral angle; the inner two are rounded, while the outermost one is large, wide and



Text-fig. 5. *Amphipholis tetracantha*. $\times 20$. a. From above. b. From below. c. Lateral view of three arm joints near disk.

operculiform. Teeth quadrangular and stout.

Dorsal arm plates rather large, oval, wider than long, as long as the corresponding arm joint, just a little in contact with, or just separated from, one another. Lateral arm plates rather low, flared outwards, those of the two sides meeting or not meeting above and meeting below. First ventral arm plate small, pentagonal, longer than wide, wider outwards than inwards, in contact with the next one. Those beyond pentagonal, with concave lateral borders, notched outer border and rounded outer lateral angles, where they are widest; longer than wide, not in contact with one another. Arm spines four in number on either side of the basal arm joints, but soon dropping to three outwards; short, conical, acute; the uppermost one is the longest and is hardly as long as the corresponding arm joint; the others are shorter. Two minute, oval, flat, leaf-like tentacle scales, of which the adradial one is slightly larger than the abradial.

Colour in alcohol: disk light yellow; arms, as well as the outer ends of radial shields, white.

This is a third Japanese species of those closely allied with *Amphipholis squamata* (DELLE CHIAJE), though the indistinctness of the primary plates, the inwardly blunt radial shields, the rather large dorsal arm plates, which are as long as the corresponding arm joint, the outwardly wider and outwardly notched ventral arm plates and the four arm spines would make the present species easy to be recognised.

These and the other Japanese species of *Amphipholis* can be distinguished as follows:

A -- Radial shields perfectly jointed in pairs.

a -- Arms three to four times as long as the disk diameter; dorsal arm plates without any streak along the median line.

b -- Radial shields acute within; dorsal arm plates small, distinctly shorter than the corresponding arm joint; ventral arm plates with convex outer border, widest at the inner lateral angles.

c -- Disk scales thin; rather indistinct from one another, so that the disk is very smooth; radial shields not very wide, about two and a half times as long as wide, the united width of each pair being nearly equal to the width of the corresponding arm base; three arm spines, of which the uppermost one is the longest, the lowest one the shortest, and the middle one the stoutest but not distinctly compressed *japonica*

cc -- Disk scales thickened along the free-margins, concave, very distinct from one another, so that the surface of disk is not very smooth;

radial shields very wide, about twice as long as wide, the united width of each pair much exceeding the width of the corresponding arm base; three, sometimes four, arm spines, of which the uppermost one is the longest, and the middle one the shortest and stoutest and distinctly compressed. *sobrina*

bb — Radial shields blunt within; dorsal arm plates rather large, as long as the corresponding arm joint; ventral arm plates with notched outer border, widest at outer lateral corners; four arm spines at arm bases, the uppermost one being the longest. *tetracantha*

aa — Arms seven to eight times as long as the disk diameter; dorsal arm plates as long as the corresponding arm joint, with a distinct white streak along the median line; ventral arm plates with notched outer border; three arm spines, of which the uppermost one is the shortest.

..... *pugetana*

AA — Radial shields divergent, being nearly or entirely separated from each other by a row of scales; three arm spines, of which the uppermost one is the shortest; tentacle scales large, the adradial one being especially the larger. *kochii*

Amphipholis pugetana (LYMAN)

CLARK, loc. cit., 1915, p. 242; MATSUMOTO, loc. cit., 1917, p. 191;

MATSUMOTO, loc. cit., 1918, p. 478.

This species is not represented in the present collection, though it has been recorded by the writer from the Aomori Bay.

Amphipholis kochii LÜTKEN

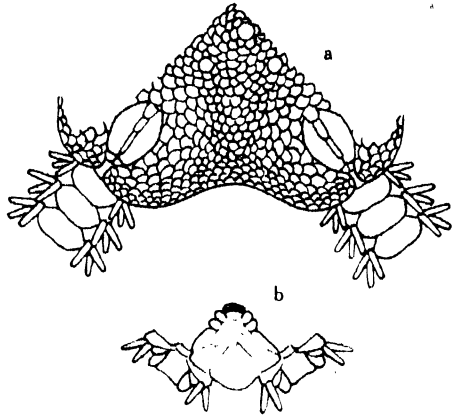
(Text-fig. 6)

CLARK, loc. cit., 1915, p. 241; MATSUMOTO, loc. cit., 1917, p. 192.

Station 17; Namiuchi. Heinai-mura; two specimens. Station 83; off Aburagawa; one specimen. Eastern shore of Yunoshima; five specimens. Off Futagojima, 18-19 fathoms; one specimen.

As already stated by the writer, this species is very variable, though is well-defined and valid. The largest one of these specimens, being that from Station 83, measures 9 mm. across the disk and some 55 mm. along the arms. In it, the armature of the oral angles and arm bases is covered over by a rather thick skin, so that the boundaries of the plates and shields are obscured, and the pair of infradental papillae are widely apart from each other, just as once illustrated by LYMAN: — Ill. Cat.

Mus. Comp. Zool., No. VIII, II, Ophiur. and Astrophyt., 1875, Pl. V, fig. 72. In another specimens, the primary plates are distinct and are located and arranged excentrically, as if the growth rate of the disk covering might have been heterogenous in different radii and interradii. The larger adradial one of the characteristically large tentacle scales is usually tongue-shaped, but is nearly round in some specimens.



Text-fig 6. *Amphipholis kochii*. $\times 8$. a. From above. b. Ventral view of one oral angle.

Amphiura sinicola, sp. nov.

(Text-fig. 7)

Station 23 A; coast of Moura; two specimens.

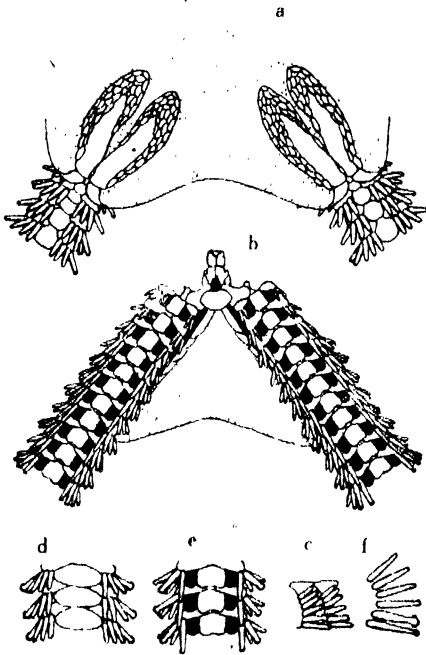
Diameter of disk 8 mm. Length of arms some 140 mm. Width of arms at base 1 mm.

Disk five-lobed, covered by a soft naked skin, except along the abradial border, inner border and inner half of adradial border of the radial shields, where a few to several rows of fine imbricating scales persist. Naked part of radial shields lance-shaped, rather long, very narrow, two fifths to one half as long as the disk radius, about four times as long as wide; their pair can be sometimes very closely set and sometimes divergent. Interbrachal ventral surfaces covered also by a soft naked skin. Genital slits long. Genital scales arranged in a row and overlapping one another.

Oral shields trapezoidal or hexagonal, with rounded corners, one and a half time as wide as long, wider within than without; madreporic shield much larger and nearly oval. Adoral shield three-lobed, with concave sides and rounded lobes, separated from each other interradially by a conspicuous depression, either meeting with each other over the first ventral arm plate or separated from each other by the same plate radially. The oral plates also embrace the just mentioned conspicuous depression, just as observed in *Amphiura vadicola* MATSUMOTO, as well as in *Ophio-*

thrix. Two oral papillae on either side of each oral angle; the infradental one is conical or peg-like and stout, while the distal one, arising almost from the inner end of the adoral shield, is weaker, abbreviatedly lanceolate, flattened and acute. Teeth quadrangular and stout.

Arms very long, about sixteen to eighteen times as long as the disk diameter. The dorsal arm plates at the base are narrow and almost



Text-fig. 7. *Amphiuva sinicola*. a. From above, $\times 8$. b. From below, $\times 8$. c. Lateral view of two arm joints near disk, $\times 8$. d. Dorsal view of three arm joints of the widest part of arm, $\times 8$. e. Ventral view of ditto, $\times 8$. f. Arm spines of one side of arm joint near disk, $\times 16$.

circular, while those of the major part of the arm beyond are transversely oval, wider than long, about one and a half time to twice as wide as long, in contact with one another to a moderate extent. Lateral arm plates not very prominent, almost concealed by the arm spines, not meeting above or below, nor in contact on the sides but separated by a naked space. First ventral arm plate quadrangular or hour-glass shaped, being constricted by the adoral shield on both sides, longer than wide. The second ventral one is squarish, slightly longer than wide, in contact with the first plate. A few plates beyond are squarish and about as wide as long. A few still beyond are squarish and wider than long. Those further beyond are pentagonal, with large and obtuse inner angle, rounded outer angles and notched outer border, and are wider than long; the successive plates are separated by very narrow

spaces, where the lateral arm plates are wedged in. Arm spines six or seven at the arm base, and five at the major middle part of the arm, peg-like, flattened, blunt, longer downwards, nearly equal to or slightly longer than the corresponding arm joint; they become more flattened outwards, the second spine from below becoming especially so and spur-shaped, with numerous minute thorns on the very much flattened end;

the lowest one and the third from below are also somewhat thorny at the tip. Tentacle pores large, unprotected.

Colour in alcohol, straw-yellow; distal parts of arms whitish.

It is quite interesting to meet with a number of species, including this new one, which are closely allied together, in Japanese waters, each having a limited range; they are *Amphiura vadicola* MATSUMOTO from the Kagoshima Gulf and Chintô, Korea, *A. ecnomiotata* CLARK from the Suruga Gulf and the Tokyô Gulf, *A. aestuarii* MATSUMOTO from Misaki and the present species from the Mutsu Bay. Two foreign species can be added here; viz. *A. phalerata* (LYMAN) from the Philippines and *A. octacantha* (CLARK) from Friday Island. Above all, the present species is most closely allied with *A. vadicola* than with any other species, the Japanese representatives being distinguished from each other as follows:

Amphiura with mostly naked disk, disk scales persisting only around the radial shields, and with large unprotected tentacle pores.

A — Five to seven arm spines, lower ones of which are thorny at the tip; dorsal arm plates in the major part of the arm very large and wide, distinctly much wider than long; oral shields wider than long.

a — Five arm spines near disk, but four more distally; those of the proximal arm joints are conical, though they become flattened outwards; dorsal arm plates very large and wide even at the arm base; arms twelve to thirteen times as long as the disk diameter. *aestuarii*

aa — Six or seven arm spines near disk, but five or six more distary, flattened; dorsal arm plates at the arm base small, narrow, round.

b — Radial shields small and very narrow, naked part being two fifth to one half as long as the disk radius and about four times as long as wide; distal oral papilla weak, abbreviatedly lanceolate, flattened, acute; dorsal arm plates at the arm base small, but not rudimentary, being in contact with one another and with the lateral arm plates; first ventral arm plate longer than wide; arms about sixteen to eighteen times as long as the disk diameter. *sinicola*

bb — Radial shields large, naked part being one half to two thirds as long as the disk radius and about three times as long as wide; distal oral papilla conical, stout, longer than the infradental one; dorsal arm plates at the arm base rudimentary, surrounded by spaces of soft naked skin; first ventral arm plate wider than long; arms exceedingly long, more than thirty times as long as the disk diameter. *vadicola*

AA — Ten arm spines with thorny tip; dorsal arm plates narrow, longer than wide; oral shields longer than wide; arms about fourteen

times as long as the disk diameter. *ecnomiotata*

Ophiothrix marenzelleri KOEHLER

(Text-fig. 8)

CLARK, loc. cit., 1915, p. 281; MATSUMOTO, loc. cit., 1917, p. 220;

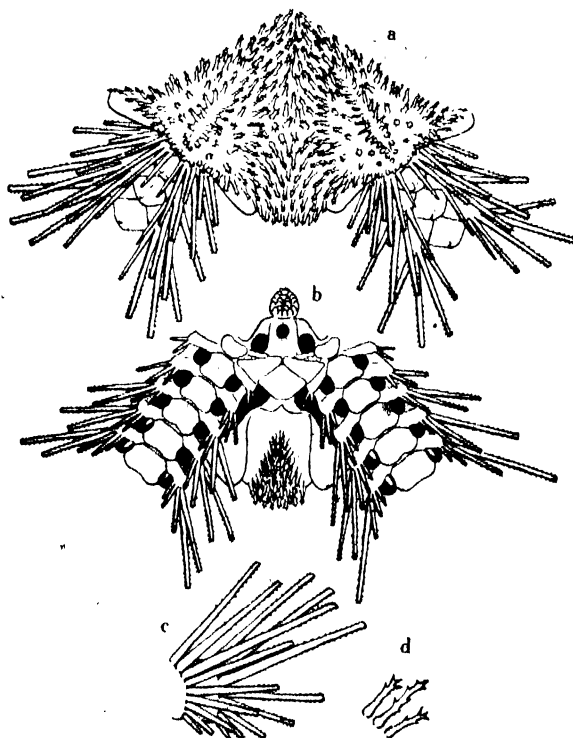
MATSUMOTO, loc. cit., 1918, p. 478.

Ophiothrix hylodes, CLARK, loc. cit., 1915, p. 273.

Station 23; Moura; one specimen.

The specimen belongs to the littoral form of the species, as distinguished by the writer, approaching however considerably to the sublittoral. The arm spines are eight or nine on either side of the basal arm joints,

only moderately widened, showing a tendency to be clavate at the tip; the third spine from above is usually the longest, being about three and a half times as long as the corresponding arm joint; the spines diminish in length both upwards and downwards. The first to third or fourth brachial tentacle pores are free of scales, while those beyond are provided with usually one, sometimes two, scales. In colour in alcohol, the disk is variegated with light brown and blue, having however a white spot at the outer end of the radi-



Text-fig. 8. *Ophiothrix marenzelleri*. a. From above, $\times 6$. b. From below, $\times 6$. c. Lateral view of two arm joints near disk, $\times 6$. d. Three disk spines or tubercles, $\times 12$.

al shields, and the arms are banded with dark brown and blue, the light-coloured streak along the dorsal median line being faintly indicated.

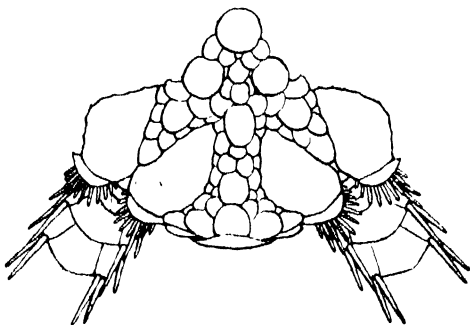
***Ophiura kinbergi* (LJUNGMAN)**

(Text-fig. 9)

CLARK, loc. cit., 1915, p. 321; MATSUMOTO, loc. cit., 1917, p. 271.

Station 19; off Tsuchiya; two specimens. Station 68; off Jōgasawa; seven specimens. Station 107; mouth of Fukuura Bay; numerous specimens.

Within the limit of Japanese waters, the present species is well-defined and appears to be fairly uniform. It shows no tendency to vary toward *Ophiura sarsii* LÜTKEN, which shows a variation toward it. Only sometimes, especially in young, the radial shields are in contact in pairs to a short extent. The Mutsu Bay and vicinity are the known northern limit of this Indo-Pacific species.

Text-fig. 9. *Ophiura kinbergi*. ×8. From above.***Ophiura sarsii* LÜTKEN**

(Text fig. 10)

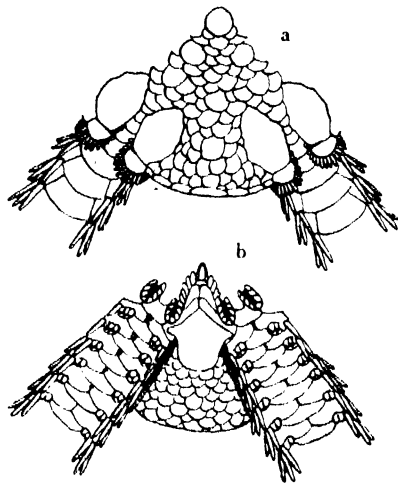
CLARK, loc. cit., 1915, p. 323; MATSUMOTO, loc. cit., 1917, p. 272;

MATSUMOTO, loc. cit., 1918, p. 479.

Station 26; off Futagajima; one specimen.

Station 43; off Ishihama-mura; abundant specimens. Off Asadokoro; numerous specimens. Off Kusodomari; nine specimens. Off Yomogida-mura; numerous specimens.

The majority of the specimens are small, including none of very large ones and a few which are some 16 mm. across the disk. A considerable number of the specimens have rather long and slender papillae of the arm combs within the limit of the present species, tending more or less to approach to *Ophiura kinbergi*. The continuation of the series of the comb

Text-fig. 10. *Ophiura sarsii*. ×4. a. From above. b. From below.

papillae is, as a rule, extended to the outer adradial border of the radial shield; and the papillae of this continuation, as well as a few immediately neighbouring ones, which arise from the comb plate, are very short and like mere granules. Besides, there occurs a series of granule-like papillae on the dorsal arm plate of the arm base inserted into the disk, arising from the opposite border of the distal continuation of the genital slit and lying under the comb papillae. The oral shields are pentagonal, with nearly linear inner sides, notched lateral sides and convex outer side; they are mostly about as long as or slightly longer than wide, but are sometimes wider than long; the lateral angles are sometimes strongly projected so as to be wing-like.

EXPLANATION OF PLATE

Plate XXI

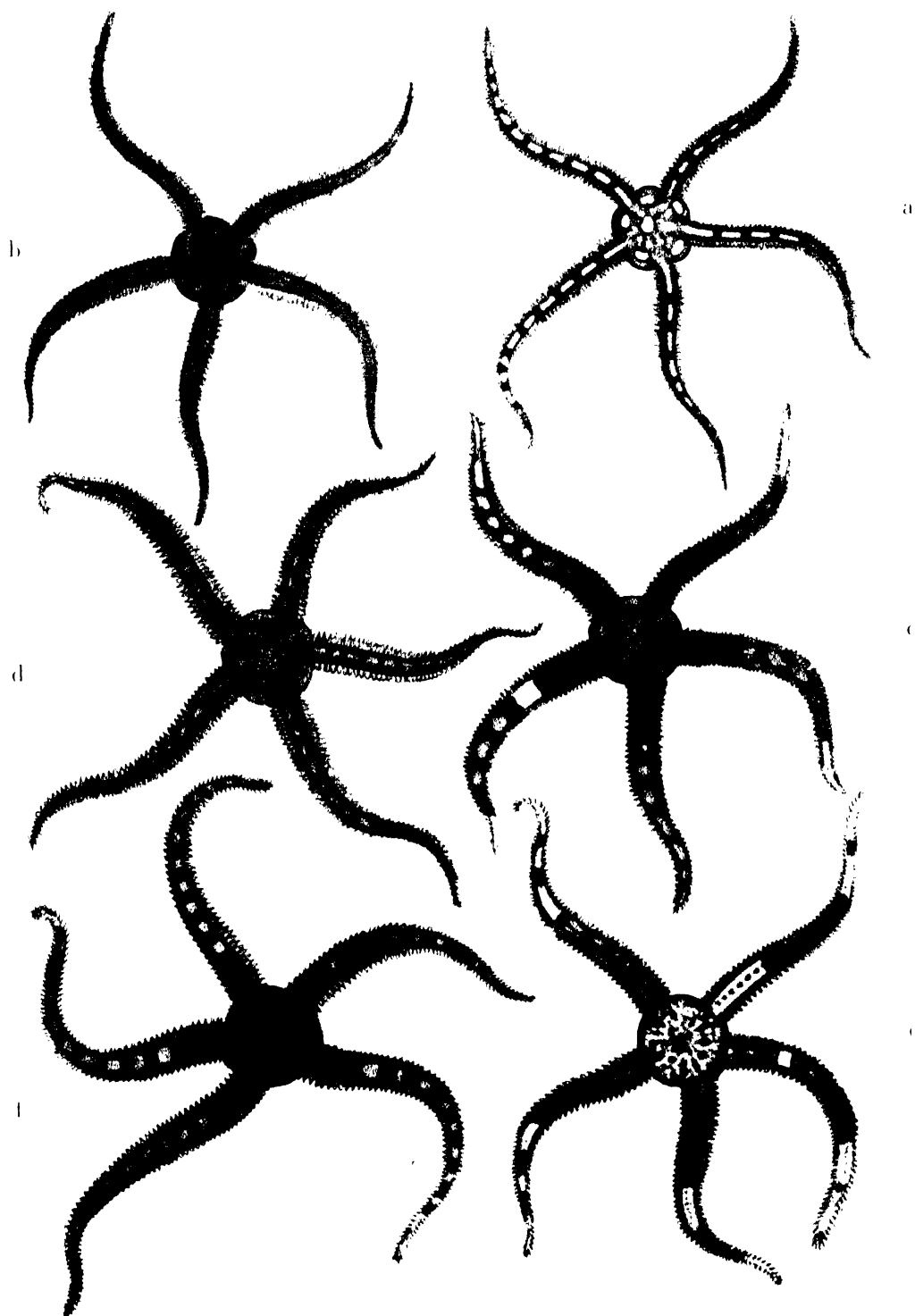
Ophiopholis mirabilis. Coloration in life in dorsal view.

Plate XXII

Ophiopholis mirabilis. Coloration in life in dorsal view.

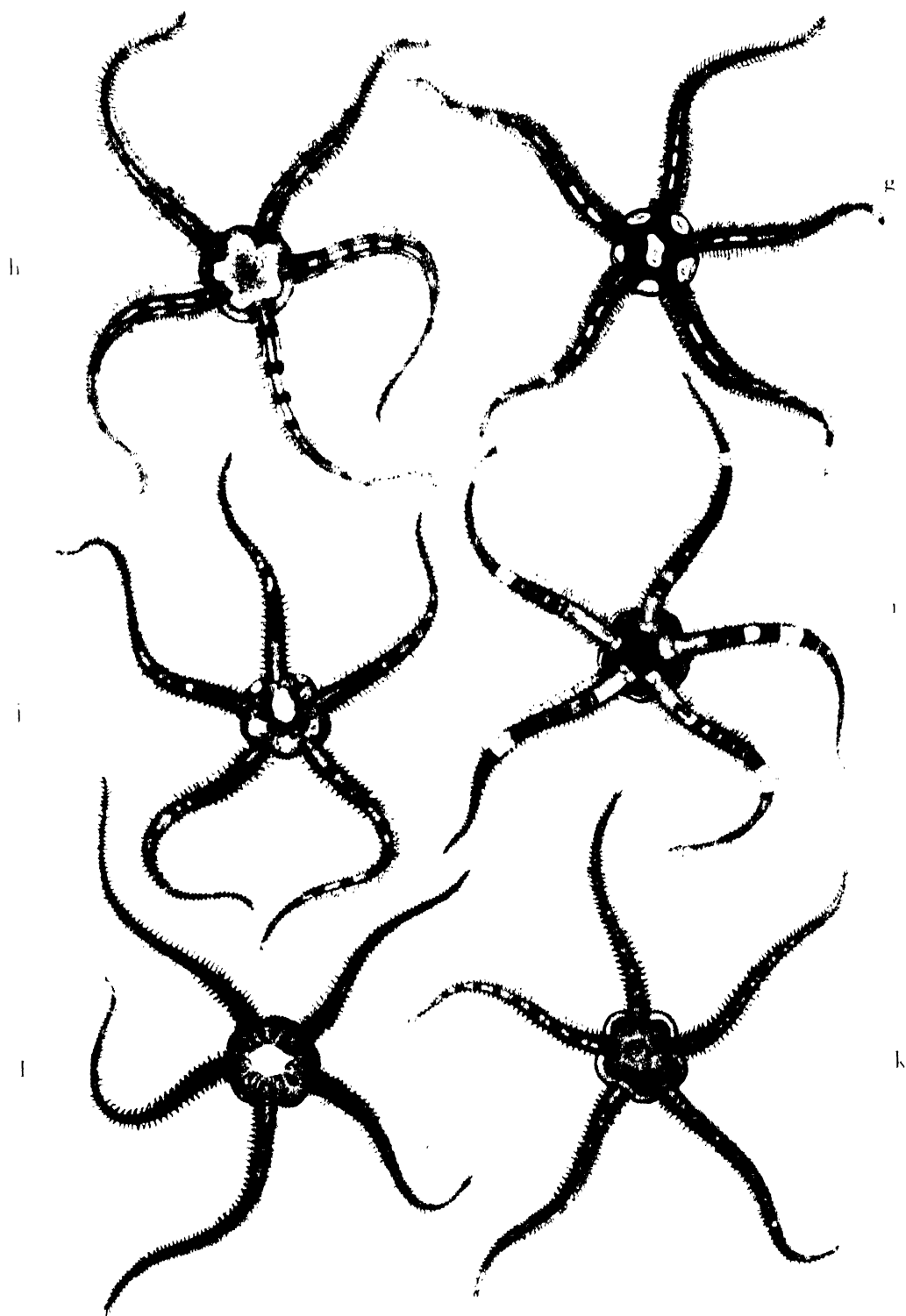
Plate XXIII

Ophiopholis mirabilis. Coloration in life in dorsal view.



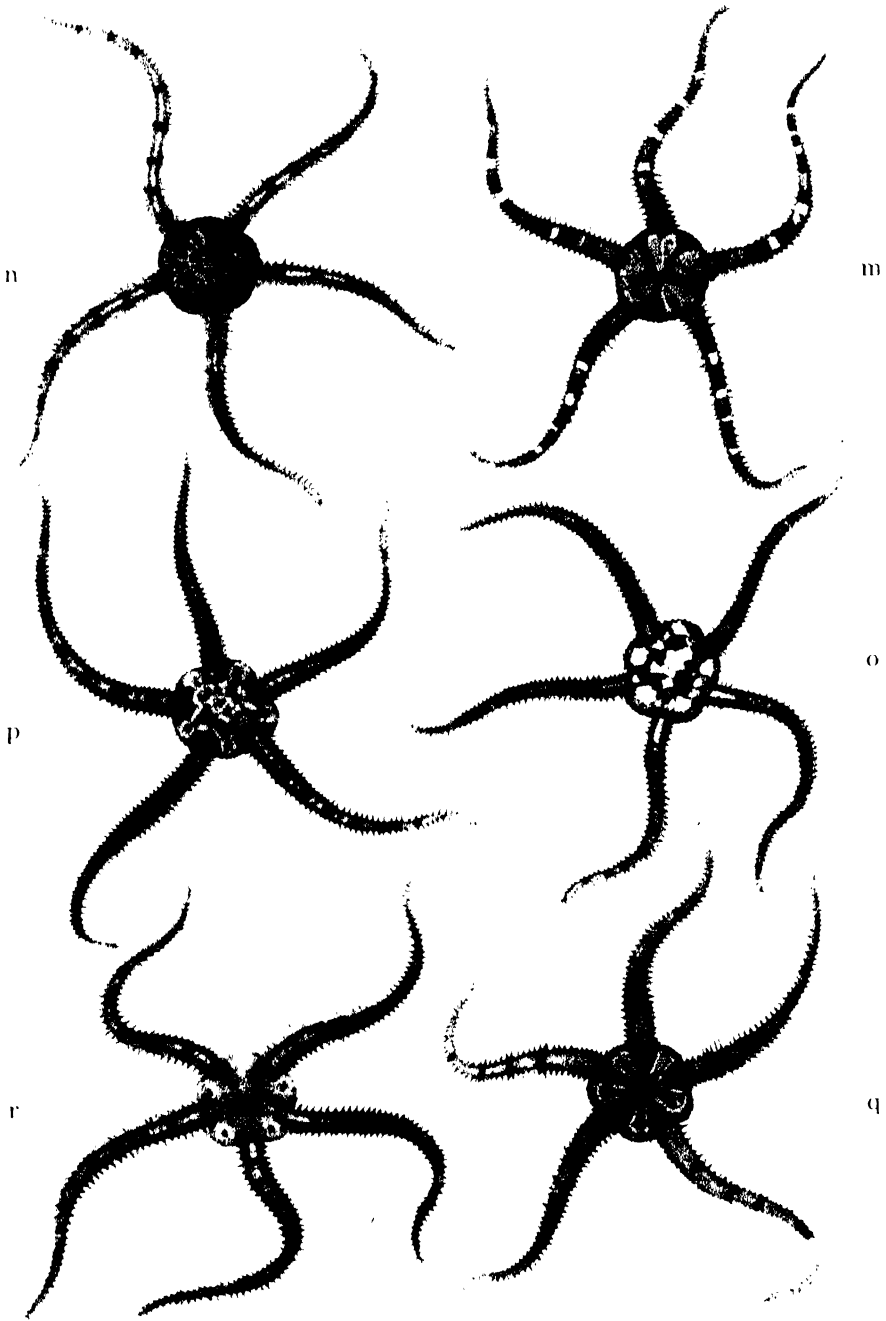
B. SAKUMA del.

H. MATSUMOTO: Ophiuroidea of Mutsu Bay.



B. SAKUMA del.

II. MATSUMOTO: Ophiuroidea of Mutsu Bay.



B. SAKUMA del.

H. MATSUMOTO: Ophiuroidea of Mutsu Bay.

MATERIALS OF THE FERTILIZATION MEMBRANE IN THE EGGS OF ECHINODERMS

By

ISAO MOTOMURA

Biological Institute, Tôhoku Imperial University, Sendai

(With Plates XXIV, XXV and 2 text-figures)

(Received April 4, 1941)

Concerning the formation of the fertilization membrane in the sea urchin's eggs, a considerable divergence of opinion has existed. One of the views is that there is a new formation; that is, the membrane is an entirely new structure formed subsequently to fertilization. The other is that the membrane is a modification of a membrane which is present before fertilization. The first view was held by HARVEY (1910), who assumed the presence of the membranogen in the egg cytoplasm, and was supported by LOEB (1913), McCLENDON (1914), and GRAY (1922). The second view is mainly based on the fact that the surface of the unfertilized egg is covered with a delicate membrane which is observable with the micro-dissection method, or in the eggs cytolysed with distilled water. CHAMBERS (1921), WHITAKER (1931), HOBSON (1932), CHASE (1935) and MOSER (1939, 1940) are of this opinion.

In 1930 A. R. MOORE showed that the eggs of sea urchins can be fertilized and caused to develop without the formation of the fertilization membrane and the hyaline membrane, if the unfertilized egg first be treated with a solution of non-electrolytes, either urea or glycerin, and then fertilized. The same result was obtained by the present writer also by using the butyric acid sea water instead of the urea solution (MOTOMURA 1934). And further, he found that the isotonic solution of urea causes the parthenogenetic membrane formation the same as the butyric acid sea water does, when the unfertilized egg is washed for a few seconds with the solution. Recently, the stimulating effect of the urea solution was described also by MOSER (1940). He found that the cortical layer granules in the unfertilized egg of *Arbacia* are broken down by treating with the urea solution as well as by the stimulation either with sperm or with other reagents such as saponin or toluol, and by the stimulation with ultraviolet radiation, direct current or with pricking. According to

him the breakdown of the cortical layer granules is a common phenomenon in the fertilization and parthenogenetic activation. And, the membrane elevation is caused by the swelling of the vacuoles which may arise as a result of the breakdown of the cortical layer granules (MOSER 1939).

The fertilization membrane of the sea urchin's egg is a relatively tough and elastic structure which cannot be identified as such prior to fertilization. The thickening of the membrane usually occurs within a few minutes after fertilization. The question is, therefore, whether the membrane is thickened by the addition of something coming from the egg cytoplasm on to it, or whether it hardens by changing its chemical or physico-chemical properties. In 1936 the writer reported that the cortical cytoplasm of the unfertilized egg of the sea urchin, *Strongylocentrotus pulcherrimus*, contains a layer of granules, which are stained intensively with Janus green B in the fresh state or in the fixed material. The granules are distinguished from mitochondria in not being stained with acid fuchsin. The present paper deals with the roll of the Janus green granules of the sea urchin's egg, as well as of the star fish's egg, on the formation of the fertilization membrane. From the observations on the behavior of the granules in the course of maturation and fertilization, the writer has come to the conclusion that the granules are one of the premembrane materials. The inhibiting effect of the urea solution or of the butyric acid sea water against the membrane forming capacity of the egg has been studied with respect to the morphological structure of the cortical cytoplasm. And the results also support the writer's opinion.

MATERIAL AND METHOD

Materials used were a sea urchin, *Strongylocentrotus pulcherrimus* A. AGASSIZ, and a star fish, *Asterina pectinifera* (MÜLLER et TROSCHEL). For the fixation of the Janus green granules CHAMPY's fluid was used. ZENKER's, BENSLEY's and FLEMMING's fluids were also useful. The paraffin sections of the fixed materials were brought through xylol, absolute and 95 per cent alcohol into N/2 NaOH aqueous solution. After twenty minutes the sections were washed with tap water, and then stained for one minute with 1 per cent aqueous solution of Janus green B (GRÜBLER) heating the slide gently over a flame. After cooling the sections were differentiated with water and alcohol, and then embedded in Canada balsam through alcohol and xylol. The Janus green granules became dark blue, the yolk granules light grey and the nucleus remained colorless. The

formalin material was also stainable in toto with a dilute aqueous solution of Janus green B, when the material was previously treated with N/2 NaOH solution. For the vital staining of the granules the egg was stained for one minute with 0.5 per cent solution of Janus green B in sea water. A dilute solution, on the contrary, could not stain the granules in the living egg.

MORPHOLOGY OF THE JANUS GREEN GRANULES IN THE SEA URCHIN'S EGGS.

A. Observations in the living eggs.

It has been reported by the writer (1936) that in the surface of the living unfertilized egg of *Strongylocentrotus pulcherrimus* a layer of small transparent granules is observable. Those granules, which measure about 0.5μ in diameter, are contained in the cortical cytoplasm, and they are not displaced by the strong centrifugal force. The density of distribution of the granules is about 120 per $100\mu^2$ (Fig. 1). They are more numerous than the pigment granules which are observable in the cortical cytoplasm by means of blue light filtered with a tricolor blue filter.

The granules are stained dark blue with concentrated solution of Janus green B (GRÜBLER) in sea water. It is noticeable that the granules cannot be stained with a dilute solution of the stain, and that the stained granules show a tendency of clinging to the neighbouring granules. The last mentioned fact suggests that the granules are semifluid or that they are a lipoid-like substance. Those granules, which are found in the cortical cytoplasm of the unfertilized egg of the sea urchin and are stainable with Janus green B, are called *Janus green granules* (JGG).

B. Observations in the material fixed and preserved in formalin.

The Janus green granules can be stained in the material fixed and preserved in formalin. The formalin material of the unfertilized egg was washed first with N/2 NaOH for one hour, next with tap water, and then stained with 0.01 per cent aqueous solution of Janus green B. With this procedure the Janus green granules are stained dark blue while the yolk granules remain unstained. This fact shows that the Janus green granules increase the affinity to the stain by the treatment with alkali. And they are distinguished also from the pigment granules by the fact that the latter are stained with basic dyes, neutral red, methylen blue etc., without being treated with the alkali solution.

C. Observations in sections.

The unfertilized egg was fixed with CHAMPY's fluid. The paraffin sections were stained with Janus green B or with Safranin O. HEIDENHAIN's iron hematoxylin did not stain the granules, while it stained yolk granules intensively. After a prolonged fixation with CHAMPY's fluid the granules take the stain very weakly, but they could be stained again with Janus green or with safranin, if the sections were washed with N/2 NaOH. The Janus green granules were stained dark blue with Janus green, yolk granules light grey, and the chromatin remained colorless.

In the unfertilized egg the Janus green granules were found only in the cortical cytoplasm, forming a layer just beneath the egg surface (Fig. 6). Although the writer has no data at present on the chemical nature of the Janus green granules, the following facts will suggest the outline; that is, 1) the granules are distinguished from chromidia by being unstained with hematoxylin and methylgreen, 2) they are not dissolved away by formalin fixation, 3) they are not mitochondria because of not being stained with acid fuchsin after the fixation with CHAMPY's fluid, and 4) they are distinguished from lipoid and from GOLGI apparatus in the point that they are blackened neither with silver nitrate nor with prolonged osmification (Fig. 2).

BEHAVIOR OF THE JANUS GREEN GRANULES IN THE COURSE OF FORMATION OF THE FERTILIZATION MEMBRANE

The fertilization membrane of the eggs of *Strongylocentrotus pulcherrimus* appears first 30 seconds after fertilization, and reaches its maximum extension 3 minutes after at 13°C. The membrane is so feeble at the beginning of its elevation that it can easily be removed by shaking the egg in a test tube. 10 minutes after it hardens perfectly. The hyaline membrane appears at about 15 minutes after fertilization.

The behavior of the Janus green granules in the course of the membrane formation is very remarkable. After fertilization the Janus green granules are discharged gradually from the egg, and parallel to this the fertilization membrane increases its affinity to the stain. After 6 minutes about half of the granules have disappeared from the cortical cytoplasm of the egg, and on the other hand the fertilization membrane is stained dark blue (Fig. 7 and 8). When the Janus green granules disappeared entirely from the cortical cytoplasm of the fertilized egg, the fertilization membrane became tough and could be stained strongly with the stain (Fig. 9).

Those facts reveal that the disappearance of the Janus green granules from the cortical cytoplasm have a close relation to the fertilization phenomena. And there must be two possibilities; that is, the granules are discharged from the egg forming a part of the fertilization membrane, or the disappearance of the granules is one of the protoplasmic changes, which has no causal relation to the membrane formation. The problem will be analysed in the following experiments.

DISTRIBUTION OF THE JANUS GREEN GRANULES IN THE IMMATURE EGG

It is a well known fact that the immature egg of the sea urchin does not form a fertilization membrane. From the observations of sections it was ascertained that the distribution of the Janus green granules in the immature egg is different from that in the mature egg with the capacity of forming a fertilization membrane. In the immature egg the granules are distributed not only in the periphery of the egg but also in the inner part of the egg (Fig. 3). In the course of the maturation division the granules moved to the egg surface (Fig. 4 and 5). And at the end of the maturation division, which is finished in the ovary, all of the granules were collected in the cortical cytoplasm (Fig. 6). In some rare cases a few of the granules remained in the endoplasm even after the completion of the maturation division. These facts will show that the movement of the Janus green granules begins at the same time as the beginning of the maturation division and is nearly finished at the completion of the division.

EFFECT OF TREATMENT WITH THE UREA SOLUTION OR WITH THE BUTYRIC ACID SEA WATER

It has been reported by the writer that the unfertilized egg of the sea urchin, *Strongylocentrotus pulcherrimus*, loses the capacity to form the fertilization membrane and the hyaline membrane, if it is previously washed with 1M solution of urea or with the sea water to which is added 6 per cent of N/10 butyric acid (MOTOMURA 1934). These two reagents are quite different in their chemical nature, but their effect is apparently the same. The problem is, therefore, to compare the effect of these two reagents on the Janus green granules of the unfertilized egg.

The unfertilized eggs were first washed with 1 M solution of urea and then were fixed with CHAMPY's fluid. In the eggs which were washed

for one minute most of the Janus green granules disappeared, and the vitelline membrane attaching on the surface of the egg was observed (Fig. 10). In the eggs which were washed for two minutes the Janus green granules, as well as the vitelline membrane, disappeared entirely (Fig. 11). In the last case the surface of the egg was covered with a thin membrane, which shows the same appearance as that limiting the surface of the cytoplasm of the fertilized egg. The time of washing necessary for the disappearance of the Janus green granules from the unfertilized egg was nearly equal to that necessary for the loss of the capacity of the membrane formation. This fact reveals that there is an intimate relation between the Janus green granules and the fertilization membrane, and that the granules and the vitelline membrane can be dissolved with the non-electrolyte solution.

On the other hand, the unfertilized egg treated with the butyric acid sea water showed a different result. Even after the prolonged treatment with the butyric acid sea water the Janus green granules did not disappear from the egg. The unfertilized egg, which was previously washed for 10 minutes with the butyric acid sea water, could be fertilized by a sperm, and it divided without forming the membranes (Fig. 12 and 13). As a result, the Janus green granules were observed in the cortical cytoplasm even after the fertilization, and consequently, the cortical cytoplasm was clearly distinguished from the newly formed surface of the blastomeres. Therefore the effect of the butyric acid sea water is quite different from that of the urea solution in that the former is not able to dissolve the Janus green granules. The vitelline membrane was equally dissolved with both solutions. The above fact shows further that the cleavage plane of the blastomeres are newly formed membranes instead of being ones covered with the extension of the old cortical cytoplasm (MOTOMURA 1935, 1940).

SOME OBSERVATIONS ON THE MECHANISM OF THE MEMBRANE FORMATION

The results of observations mentioned above are summarized as follows:

- 1) The Janus green granules disappear just after fertilization, and parallel to this the fertilization membrane is thickened gradually. The staining reaction of the granules and the fertilization membrane reveals the possibility that the granules are discharged from the egg and precipitate on the vitelline membrane.
- 2) The immature egg, in which the layer of the Janus green granules is not yet formed at the periphery of the egg, does

not form the fertilization membrane. 3) The formation of the fertilization membrane is inhibited either by dissolving the granules with the isotonic urea solution, or by inhibiting the discharge of the granules from the egg with the butyric acid sea water. Thus, all these facts suggest the possibility that the Janus green granules are one of the premembrane materials of the fertilization membrane. The hyaline membrane has, on the contrary, no close relation to the granules. As mentioned above the hyaline membrane first appears 15 minutes after fertilization. And the disappearance of the granules is completed much earlier than the formation of the hyaline membrane. The hyaline membrane is not stained intensively with Janus green, while the fertilization membrane is stained dark blue. Thus, the hyaline membrane has less relation to the Janus green granules. The fact that the formation of the fertilization membrane is completed within the first 10 minutes after fertilization, will be ascertained by the following facts.

A. Centrifuged eggs.

The gelatinous envelope of the unfertilized egg was removed by gentle centrifuging, and a large number of eggs were fertilized in a small vessel. When the fertilization membrane became hard, the eggs clung to each other fusing the surface of the fertilization membrane of each eggs. This fact shows the difference in the nature of the fertilization membrane prior and after fertilization.

B. Clinging of the fertilized eggs.

The fertilization membrane was removed by shaking the eggs three minutes after fertilization. The eggs then settled on the bottom of the vessel, and remained there for a few minutes. The eggs clung to each other forming a mass of eggs. They could not be separated again with calcium-free sea water. The same tendency was observed also in the eggs shaken in a small amount of calcium-free sea water at the stage of the feeble fertilization membrane, if the egg suspension was not continuously washed with the same medium. In both cases the eggs were cemented closely with a material insoluble in calcium-free sea water. The cementing substance is secreted for about 10 minutes, from the beginning of the elevation of the vitelline membrane to the completion of the fertilization membrane. Before the hardening occurs the cementing substance is soluble in calcium-free sea water, while after the hardening it become insoluble. The hardening of the cementing substance, as well as the fertilization membrane, occurs when calcium ions are present in the medium. The duration of the time of the secretion of the cementing substance coincides

clearly with that of the disappearance of the Janus green granules from the cortical cytoplasm of the egg.

C. An experiment of the separation of the blastomeres.

As long as the Janus green granules are present in the cortical cytoplasm, the egg shows the capacity of forming a precipitation membrane on its surface, even in the egg in which the fertilization membrane was removed mechanically. This is shown in the following experiment. The fertilization membrane was removed by shaking the eggs in calcium-free sea water. The eggs were then washed for 15 minutes changing the calcium-free sea water repeatedly, and after this they were cultured in ordinary sea water. The hyaline membrane was formed as in the normal egg. At the two-cell stage the eggs were again put into calcium-free sea water. The hyaline membrane was so easily dissolved in the last mentioned medium that the blastomeres could be separated by gently stirring the medium. Contrary to this, in the eggs in which the fertilization membrane was removed by shaking in sea water instead of in calcium-free sea water, the blastomeres were not separated in calcium-free sea water. The difference between the above mentioned two cases is only that in the former case the eggs were washed carefully at the time of the membrane formation, while in the latter case the eggs remained in sea water through the entire time. And it is possible to assume in the latter case that a precipitation membrane, which packs the blastomeres, was formed on the surface of the eggs in contact with calcium ions in the surrounding medium. This precipitation membrane was observed in the furrow region forming a bridge of membrane between two blastomeres.

Those facts show that the Janus green granules are the materials which are transformed to an insoluble substance by coming in contact with calcium ions. Thus, the hardening of the fertilization membrane is accomplished by adding the precipitating substance, the Janus green granules, on the inner surface of the vitelline membrane.

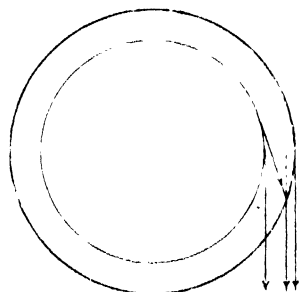
POSITION OF THE JANUS GREEN GRANULES WITHIN THE CORTICAL
CYTOPLASM OF THE FERTILIZED AND UNFERTILIZED EGGS

In the foregoing chapter it has been described how the Janus green granules migrate to the egg periphery during the course of maturation division. The position of the layer of the Janus green granules in relation to other structures will be discussed in this chapter. It has been reported by the writer that the rigid cortical cytoplasm, immovable by strong

centrifugal force, is present in the eggs of some sea urchins (MOTOMURA 1934, 1935). In the eggs of *Strongylocentrotus pulcherrimus* the cortical cytoplasm is characterized by the presence of yellow pigment granules, which become visible in blue light filtered with a blue filter of the tricolor photograph. In the unfertilized egg it seems as if the pigment granules are closely adhering to the inner surface of the morphological protoplasmic surface film when the largest optical section of the egg is observed (Fig. 14). And after the fertilization, a finely granular zone, that is, the extra-granular zone, becomes visible between the protoplasmic surface film and the layer of the pigment granules (Fig. 16). The problem is, therefore, to discover the position of the layer of the Janus green granules in relation to other structures of the cortical cytoplasm, and to discover the mechanism of the appearance of the extra-granular zone in the fertilized egg.

The unfertilized eggs were put into a mixture of 7 parts of 1 M solution of saccharose and one part of sea water, and were observed in this medium. Now, a layer of colorless granules lying between the layer of the pigment granules and the protoplasmic surface film became visible (Fig. 15). This layer was similar to the extra-granular zone of the fertilized egg in its appearance and position. And moreover, the position of the colorless granules exactly coincided with that of the Janus green granules stained in sections or in the living unfertilized egg. The layer of the colorless granules was, therefore, identical with that of the Janus green granules in the unfertilized egg. And further, it suggested that the extra-granular zone of the fertilized egg had some relation to the layer of the colorless granules.

And, the reason why the layer of the Janus green granules of the unfertilized egg is invisible in sea water, can be easily explained in that the refractive index of the layer is relatively high in comparison with sea water. As shown in Text-fig. 1 the thickness of the wall of a hollow glass sphere is apparently much reduced when it is observed in air in comparison with water. In the same way the thickness of the layer of the Janus green granules is apparently less in sea water than in the saccharose solution, because the refractive index of the saccharose solution is higher than sea water. And by mixing the saccharose solution with



Text-fig. 1. Diagram to show the decrease of the apparent thickness of the wall of a glass vessel in the medium of a low refractive index.

sea water in different ratios the refractive index of the medium can easily be changed without changing its osmotic pressure. Only when the refractive index of the external medium is equal to that of the layer of the Janus green granules, will an accurate measurement of the thickness of the layer be possible. Unfortunately the measurement of the refractive index of the granules, consequently the measurement of the thickness of the layer, could not be accomplished by the writer, because the external surface of the layer of the granules was covered with the protoplasmic surface film, which was supposed to be different in its refractive index. The thickness of the layer in the saccharose solution measured 1.3μ in the largest optical section.

As mentioned above, the Janus green granules are discharged in the fertilized egg. The loss of the granules of a high refractive index will bring a decrease of the refractive index of the layer. And it is possible that the layer of the Janus green granules becomes visible by decreasing its refractive index. Thus, the extra-granular zone will be the layer which has lost the Janus green granules in the course of the membrane formation. This assumption is also ascertained by the fact that the thickness of the layer of the Janus green granules in sections is nearly equal to that of the extragranular zone measured by DAN and DAN. And further, the inward movement of the pigment granules advocated by DAN and DAN is, according to the writer's opinion, nothing but an optical illusion caused by the change of the refractive index in the layer of the Janus green granules.

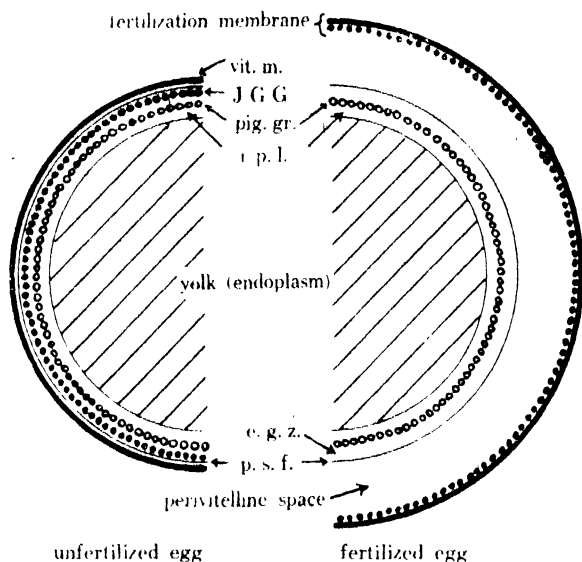
The unfertilized egg thus treated with the saccharose sea water did not lose the capacity of forming the fertilization membrane in sea water. This fact shows that the mixture of 1 M saccharose solution and sea water did not stimulate the egg. And accordingly, the visibility of the layer of the Janus green granules in the medium is caused only by the optical condition rather than the changes in the parthenogenetically stimulated egg.

In the eggs, which were treated for 10 minutes with the butyric acid sea water and then fertilized in sea water, the extra-granular zone was not visible in sea water even when the eggs were in the cleavage stage. The sections of those eggs showed, as mentioned above, that a distinct layer of the Janus green granules still remained in the cortical cytoplasm. And it is possible to observe from this fact that the visibility of the extragranular zone in the fertilized egg is caused by the loss of the Janus green granules, and that if the granules are present the zone does

not appear. And moreover, it is showed that the secretion of the Janus green granules is not essential to the process of fertilization and cleavage.

In the centrifuged, unfertilized egg a layer of cytoplasm was observed between the layer of the pigment granules and the lipid layer at the centripetal side. It is supposed that this layer is a part of the cortical cytoplasm, which has a high rigidity in comparison with the endoplasm. In the cortical cytoplasm, therefore, the following four layers were recognized; they are, 1) the inner protoplasmic layer, 2) the pigment granule layer, 3) the Janus green granule layer, 4) the proroplasmic surface film (Text-fig. 2). The surface of the protoplasmic surface film is covered with the vitelline membrane and the gelatinous envelope.

In short, the layer of the Janus green granules in the unfertilized egg lies in the periphery just beneath the protoplasmic surface film. The extragranular zone in the fertilized egg is identical with the layer of the Janus green granules in the unfertilized egg. The difference is that in the former the granules have disappeared in the course of the formation of the fertilization membrane.



Text-fig. 2. Diagram of the structure of the sea urchin's egg; left half, unfertilized egg; right half, fertilized egg. e. g. z. extra-granular zone, i. p. l. inner protoplasmic layer (I), JGG Janus green granule layer (III), pig. gr. pigment granule layer (II), p. s. f. morphological protoplasmic surface film (IV), vit. m. vitelline membrane.

OBSERVATIONS IN THE EGGS OF *ASTERINA PECTINIFERA*

The egg of the star fish, *Asterina pectinifera* (MÜLLER et TROSCHERL),

were fixed with BENSLEY's fluid for 24 hours. The paraffin sections of the eggs were stained with Janus green B. In the ovarian egg, which contains a large germinal vesicle, a layer of the Janus green granules was observed in the periphery of the egg (Fig. 17). The granules adhered closely to the inner surface of the morphological protoplasmic surface film. The density of the granules was not large in comparison with that in the sea urchin's egg. The germinal vesicle of the egg begins to disappear, if the follicle epithelium of the egg is removed by shaking the egg in sea water, and 30 minutes after shaking it became invisible. The layer of the Janus green granules was not affected by the disappearance of the germinal vesicle (Fig. 18).

In the fertilized egg the Janus green granules disappeared gradually from the egg periphery, and 5 minutes after fertilization most of them were discharged (Fig. 19). In some cases the secreted granules were observed still remaining on the external surface of the protoplasmic surface film. In short, the process of discharge of the Janus green granules in the star fish's egg in the course of membrane formation was quite similar to that in the sea urchin's eggs. But on the other hand, in the star fish's eggs the migration of the granules to the egg periphery had been completed already prior to the dissolution of the germinal vesicle.

INTERPRETATION OF THE RESULTS

Based on the results of observations mentioned above an outline of the mechanism of formation of the fertilization membrane can be summarized as follows. In the eggs of the sea urchin and of the star fish two elements take part in the membrane formation. The one element is the Janus green granules and the other is the vitelline membrane. In the normal fertilization the Janus green granules are secreted from the egg between the vitelline membrane and the protoplasmic surface film, and they swell there forming a large space, the perivitelline space. This is the process of the membrane elevation in the early stage of the normal fertilization. After this the precipitation stage follows. That is, the secreted granules come into contact with sea water at the inner surface of the elevated vitelline membrane, and they precipitate. Thus, the elevated vitelline membrane, hardens gradually with the addition of the precipitating substance. The process of formation of the fertilization membrane is the overlapping of two processes, the elevation of the vitelline membrane and the precipitation of the Janus green granules on it.

The elevation of the vitelline membrane alone can be induced experimentally, if the unfertilized eggs of the sea urchin are first washed for seven seconds with 1 M urea solution of pH 8.3, and then transferred to M/2 NaCl solution (MOTOMURA 1938). In this case the membrane is elevated as in the ordinary sea water, but it does not thicken, because the medium does not contain the ions necessary for the precipitation of the Janus green granules.

The elevation of the membrane does not occur, when the vitelline membrane had been previously removed by shaking the egg with sea water, notwithstanding the presence of both calcium ions and the secreted substance. In this case the precipitation reaction is continued at the surface of the protoplasmic surface film. And as a result the egg is covered with a tough membrane adhering on the egg surface. And thus, the blastomeres cannot be separated by dissolving the hyaline membrane. Contrary to this, when both the vitelline membrane and the Janus green granules are removed by shaking with calcium-free sea water, and by repeated washing with the last medium, the blastomeres can be easily separated. The precipitation of the secreted substance from the egg can be ascertained also by the fact that the eggs show the tendency of clinging to each other for about 10 minutes after fertilization, if the fertilization membrane is removed. All these facts reveals that the fertilization membrane is composed of at least two sorts of materials, the vitelline membrane and the Janus green granules, and that either of the alone is not able to form the tough fertilization membrane.

As to the structure of the cortical cytoplasm the writer finds no fundamental difference between the fertilized and the unfertilized eggs, except that in the former the Janus green granules are secreted in the normal fertilization. In the unfertilized egg the cortical cytoplasm is composed of four layers; they are, 1) the inner protoplasmic layer, 2) the pigment granule layer, 3) the Janus green granule layer, and 4) the morphological protoplasmic surface film. In the fertilized egg the first, the second and the fourth layers remain unchanged. But the third layer shows a remarkable change in the course of the membrane formation. Namely, almost all of the Janus green granules are discharged in the perivitelline space causing the elevation of the vitelline membrane and the decrease of the refractive index of the layer. As a result, the third layer becomes visible in the fertilized egg as the extra-granular zone described by DAN and DAN. Therefore, according to the writer's opinion, the extra-granular zone is nothing but the Janus green granule layer with its refractive

index decreased. This interpretation is based on the above mentioned fact that the Janus green granule layer can easily be observed by increasing the refractive index of the surrounding medium.

DISCUSSION

In 1935 CHASE showed that the surface of the sea urchin's egg is covered with a thin layer of semi-fluid material, which is comparable with the vitelline membrane of the star fish's egg. This structure is observable also in the egg of *Strongylocentrotus pulcherrimus* (Fig. 10 and 15). By stimulating with the urea solution the membrane can be easily elevated even in the isotonic solution of NaCl and of KCl, but it does not harden in those media (MOTOMURA 1938). Accordingly, although the presence of the membrane, the vitelline membrane, cannot be questioned, there is no positive proof that the membrane becomes hard in contact with oxygen or with the external medium alone. But it revealed at least that other materials are necessary for hardening the fertilization membrane.

According to HARVEY's opinion, the fertilization membrane is newly formed with a membrane forming substance, the membranogen, which is secreted from the egg by increasing the permeability in the course of fertilization and hardens to the membrane in contact with sea water (HARVEY 1910). As shown in the above description the Janus green granules alone were not able to form the elevated membrane in the eggs from which the vitelline membrane was previously removed.

GRAY (1922) stated that the fertilization membrane is formed by the precipitation of the electro-negative colloid, which is set free from the vitelline membrane consisting of a lipoid substance, in contact with the electro-positive colloid of the gelatinous envelope. Thus, according to him, the elements necessary for the membrane formation are the gelatinous envelope and the membranogen, which is enclosed in the vitelline membrane. The gelatinous envelope of the egg of *Strongylocentrotus pulcherrimus* can be easily removed by gentle centrifuging. And this procedure does not disturb the membrane formation. Moreover, the Janus green granules are not contained in the vitelline membrane, but they are included in the egg cytoplasm. The writer's findings, therefore, cannot be explained with GRAY's assumption.

The Janus green granules in the eggs of *Strongylocentrotus pulcherrimus* and of *Asterina pectinifera* seemed to be comparable with the cortical layer granules of MOSER's observation in *Arbacia*. The average

diameter of the Janus green granule in the living egg of *St. pulcherrimus* is 0.5μ , and that of the cortical layer granule in *Arbacia* is 0.79μ according to MOSER (1939). The cortical layer granules in the unfertilized egg of *Arbacia* break down within ten seconds when the egg is fertilized by a sperm or stimulated with molar solution of urea. Those facts show the similarity between the Janus green granules and the cortical layer granules, except that in the latter the reaction to the stain has not yet been studied.

As to the mechanism of the membrane formation, on the other hand, the writer's interpretation of the roll of the Janus green granules is different from MOSER's opinion. He (MOSER 1939) says, "It is conceivable that the cortical layer granules, when they break down, give rise to vacuoles which coalesce beneath the plasma membrane, so that the film on that side of the vacuole toward the egg becomes the new egg surface, while the vacuolar film toward the periphery of the egg becomes the inner margin of the fertilization membrane. The area between these two films would then represent the perivitelline space. As mentioned above, the Janus green granules are secreted from the egg between the protoplasmic surface film and the vitelline membrane. As shown in Fig. 3 and 4 the not yet discharged granules always stay in the egg cytoplasm, instead of staying in the perivitelline space. This fact shows that the Janus green granules never form the vacuoles before being secreted from the egg. And the precipitation reaction of the Janus green granules will occur only at the inner surface of the vitelline membrane, and it will not occur, on the other hand, at the surface of the egg cytoplasm, because the precipitating substance comes in contact with sea water only at the inner surface of the vitelline membrane. Within the perivitelline space the ions necessary for the precipitation will be soon exhausted by the reaction of a small amount of the Janus green granules, and thus, the perivitelline space will be filled with the colloidal solution of the granules at the beginning of the membrane formation. Therefore, the fertilization reaction of the Janus green granules is the secretion, and it is not the vacuolization at the inner side of the plasma membrane."

The stimulating effect of molar solution of urea was first found by the writer in the eggs of some Japanese sea urchins and was ascertained by MOSER in an American species. The effect of the urea solution shows a remarkable parallelism with that of the butyric acid sea water on the unfertilized eggs of the sea urchins. A treatment for a short time either with the urea solution or with the butyric acid sea water

causes the parthenogenetic membrane formation. If the action of these solutions is longer continued the egg will not elevate the membrane, although it can be seminated. In the case of the strongest effect the capacity of forming the membrane is lost, and the connections between the blastomeres become loose. Namely, the responses of the eggs to those reagents are simply related to the strength of effect irrespective of the sort of reagents used. As described above, the response of the Janus green granules to the urea solution is different from that to the butyric acid sea water. The granules completely disappear when treated with the former solution, while they remain in the egg when treated with the latter. This fact shows that the discharge of the Janus green granules is not a necessary condition of further development. Thus, the egg can be seminated without discharging the granules, and moreover it can cleave. It is possible to assume, therefore, that the discharge of the Janus green granules is one of the responses in the normal fertilization, and that it is not the essential first step preceeding the further development. In this connection it will be interesting to compare the responses of the cortical layer granules in the egg of *Arbacia* with that of the Janus green granules. Unfortunately it has not been reported in *Arbacia* as far the writer is aware.

The structure of the sea urchin's egg assumed from the results of of the above observation is different from GRAY's, MOSER's and DAN's opinions. It has been shown by the writer that the Janus green granules are contained in the egg cytoplasm, while GRAY assumed the electro-negative colloid to be in the vitelline membrane. And in the egg of *Strongylocentrotus pulcherrimus* the vitelline membrane is elevated by the colloid osmotic pressure of dissolved Janus green granules, while in the egg of *Arbacia* the plasma membrane is elevated as a result of vacuolization of the cortical layer granules according to MOSER's opinion. And finally, DAN and DAN (1940) are of the opinion that the pigment granules of the egg of *St. pulcherrimus* lie at the inner surface of the protoplasmic surface film before fertilization, and that they migrate inward from the surface by 1.5μ leaving a finely granular zone, the, extra-granular zone, of the same width at the cell periphery. As mentioned above, the pigment granules are not in direct contact with the protoplasmic surface film in the unfertilized egg, but there is a layer of the Janus green granules lying between the pigment granule layer and the protoplasmic surface film. The Janus green granule layer is invisible in sea water, but it become observable, if the egg is put into a medium of a

high refractive index. Therefore, the apparent absence of the Janus green granule layer in sea water is an optical illusion caused by the difference of the refractive index between the layer and sea water. Accordingly, it will be clear that the extra-granular zone of the fertilized egg is not a new structure formed as a result of the inward migration of the pigment granules, but it is a modification of the refractive index of the Janus green granule layer.

SUMMARY

1) The morphological characters of the Janus green granules in the eggs of echinoderms were described.

2) The migration of the granules to the egg periphery is finished in the course of maturation division of the egg of a sea urchin, *Strongylocentrotus pulcherrimus*. In the egg of *Asterina pectinifera* the layer of the Janus green granules is formed before the beginning of the maturation division.

3) The Janus green granules are secreted by the egg in the course of the formation of the fertilization membrane. This continues for ten minutes after fertilization.

4) The Janus green granules of the unfertilized egg are dissolved with molar solution of urea, while they are not dissolved with the butyric acid sea water. The egg developed without secreting the granules, when it was treated for ten minutes with the butyric acid sea water prior to the fertilization.

5) The mechanism of formation of the fertilization membrane was discussed.

6) The fertilization membrane is composed of two sorts of materials, the vitelline membrane and the Janus green granules. The former is elevated from the egg surface by the colloid osmotic pressure of the secreted portion of the Janus green granules. And the latter form the precipitation membrane at the inner surface of the elevated vitelline membrane.

7) The cortical cytoplasm of the unfertilized egg of the sea urchin is composed of four layers, 1) the inner protoplasmic layer, 2) the pigment granule layer, 3) the Janus green granule layer, and 4) the morphological protoplasmic surface film. In the fertilized egg the first, the second and the fourth layers remain unchanged, while the third layer decreases its refractive index by secreting the highly refractive Janus green granules

in the course of the normal fertilization.

8) The extra-granular zone of the fertilized egg is the remaining portion of the third layer, the Janus green granule layer.

LITERATURE CITED

- CHAMBERS, R. 1921. Microdissection studies. III. Some problems in the maturation and fertilization of the echinoderm egg. Biol. Bull. **41**, 318.
- CHASE, H. Y. 1935. The origin and nature of the fertilization membrane in various marine ova. Biol. Bull. **69**, 159.
- DAN, K. and J. C. DAN, 1940. Behavior of the cell surface during cleavage. III. On the formation of new surface in the eggs of *Strongylocentrotus pulcherrimus*. Biol. Bull. **78**, 486.
- GRAY, J. 1922. A critical study of the facts of artificial fertilization and normal fertilization. Quart. Journ. Micr. Sci. **66**, 419.
- HARVEY, E. N. 1910. The mechanism of membrane formation and other early changes in developing sea-urchins' eggs as bearing on the problem of artificial parthenogenesis. Journ. exper. Zool. **8**, 355.
- HOBSON, A. D. 1932. On vitelline membrane of the egg of *Psammechinus milialis* and of *Teredo norvegica*. Journ. exper. Biol. **9**, 93.
- LOEB, J. 1913. Artificial parthenogenesis and fertilization. Chicago.
- MCCLENDON, J. F. 1914. On the nature and formation of the fertilization membrane of the echinoderm egg. Intern. Zeits. f. phys.-chem. Biol. **1**, 163.
- MOORE, A. R. 1930. Fertilization and development without membrane formation in the eggs of sea urchin, *Strongylocentrotus purpuratus*. Protoplasma. **9**, 9.
- MOSER, F. 1939. Studies on a cortical layer response to stimulating agents in the *Arbacia* egg. I Response to insemination. Journ. exper. Zool. **80**, 423.
- , 1940. Studies on a cortical layer response to stimulating agents in the *Arbacia* egg. III. Response to non-electrolyte. Biol. Bull. **78**, 68.
- MOTOMURA, J. 1934. On the mechanism of fertilization and development without membrane formation in the sea urchin egg, with notes on a new method of artificial parthenogenesis. Sci. Rep. Tōhoku Imp. Univ. Biol. **9**, 33.
- , 1935. Determination of the embryonic axis in the eggs of amphibia and echinoderms. Sci. Rep. Tōhoku Imp. Univ. Biol. **10**, 211.
- , 1936. Note on the cytoplasmic structure of the egg of a sea urchin, *Strongylocentrotus pulcherrimus* (A. AGASSIZ). Zool. Mag. (Japan). **48**, 753.
- , 1938. Effect of some salt solutions on the parthenogenetic membrane formation of sea urchin eggs. Sci. Rep. Tōhoku Imp. Univ. Biol. **13**, 85.
- , 1940. Studies of cleavage. I. Changes in surface area of different regions of eggs of a sea urchin in the course of the first cleavage. Sci. Rep. Tōhoku Imp. Univ. Biol. **15**, 121.
- WHITTAKER, D. M. 1931. On the conduction of the cortical change at fertilization in the starfish egg. Biol. Bull. **60**, 23.

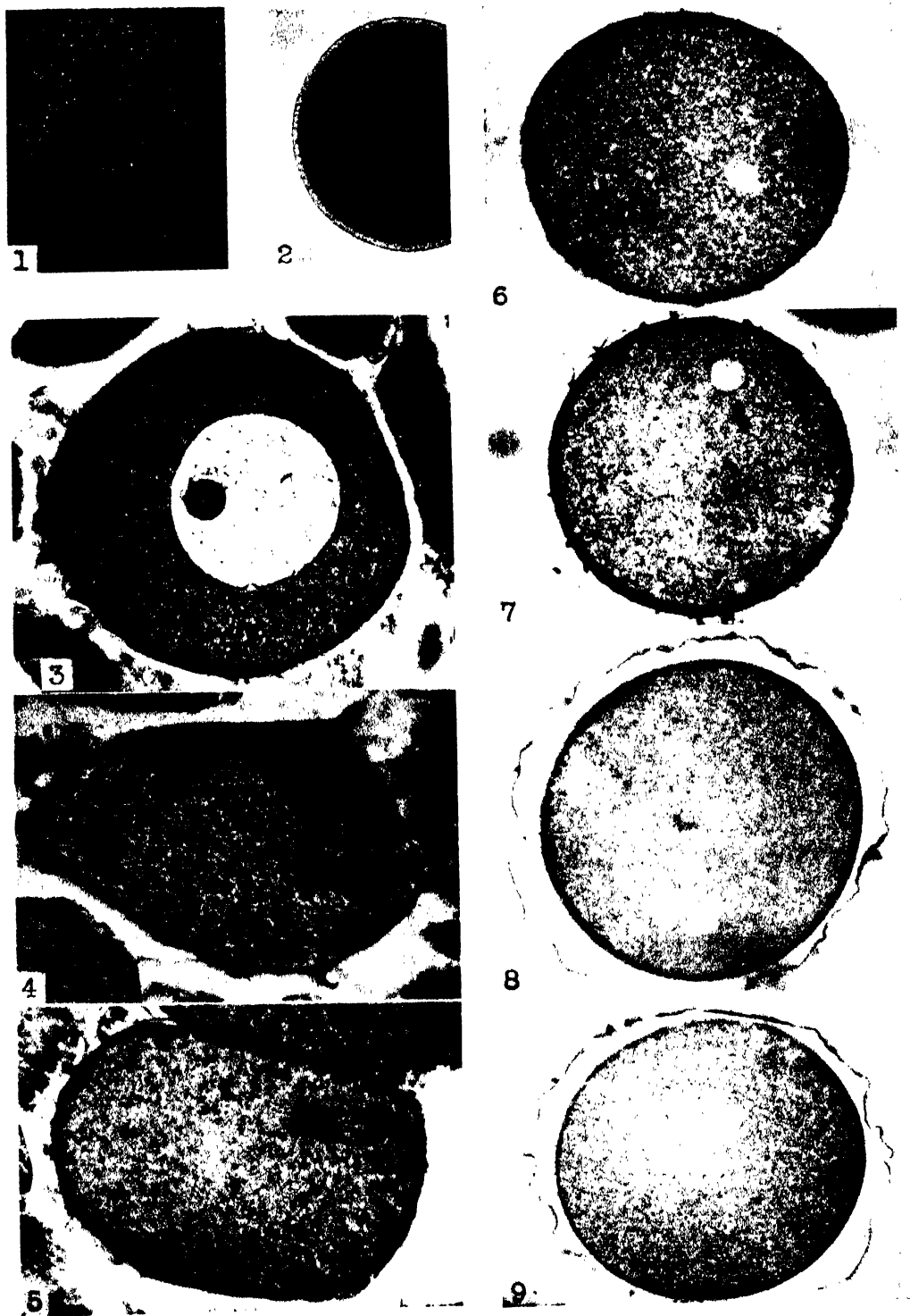
EXPLANATION OF PLATES

Plate XXIV

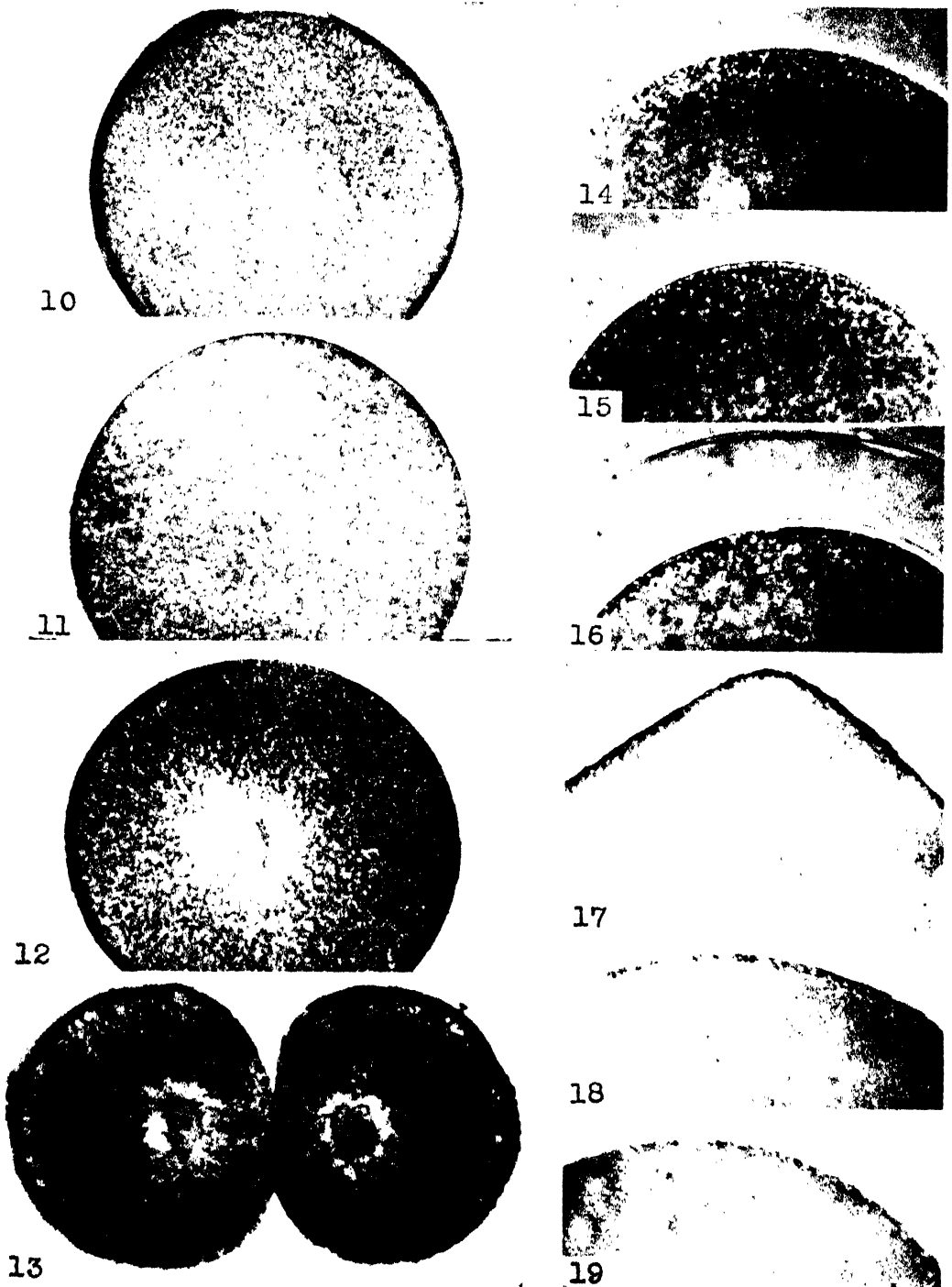
- Fig. 1. Cortical cytoplasm of a living unfertilized egg of *St. pulcherrimus*, focused on the upper surface to show the distribution of the Janus green granules (JGG) (white granules). $\times 630$.
- Fig. 2. Unfertilized egg of the sea urchin, fixed with OsO_4 and photographed in toto. The thickness of the JGG layer increased by its swelling. Transparent layer at the surface is the JGG layer. $\times 290$.
- Fig. 3-5. Maturation of the sea urchin's eggs showing the migration of JGG to the egg periphery. $\times 630$. Fig. 3, Immature egg. Fig. 4 and 5, metaphase of the first maturation division.
- Fig. 6-9. Secretion of the JGG in the course of formation of the fertilization membrane. $\times 630$. Fig. 6, unfertilized egg. JGG are stained black at the periphery of the egg. Fig. 7 and 8, Six minutes after fertilization. JGG are reduced by secretion. Fig. 9. Ten minutes after fertilization. JGG have disappeared completely.

Plate XXV

- Fig. 10 and 11. Unfertilized eggs washed with molar solution of urea. In both cases JGG disappeared. $\times 700$. Fig. 10, Egg washed for one minute. Fig. 11, Egg washed for 2 minutes.
- Fig. 12 and 13. Eggs washed with the butyric acid sea water for 10 minutes and then fertilized. $\times 700$. JGG remained in the eggs. Fig. 12, 60 minutes after fertilization. Fig. 13, 2 hours after fertilization. JGG are distributed only in the cortical cytoplasm, while in the newly formed cell surface they are not found.
- Fig. 14-16. The largest optical sections of living eggs to show the structure of the cortical cytoplasm. $\times 630$. Fig. 14, Unfertilized egg photographed in sea water. JGG layer is invisible. Fig. 15, Unfertilized egg photographed in a mixture of 1 M saccharose (7 parts) and sea water (1 part). JGG layer appeared. Fig. 16, Fertilized egg photographed in sea water. The extragranular zone appeared.
- Fig. 17-19. Eggs of *Asterina pectinifera*, fixed with BENSLEY's solution and stained with Janus green. $\times 630$. Fig. 17, Ovarian egg with the germinal vesicle. Fig. 18, Egg at the metaphase of the first maturation division. Fig. 19, Fertilized egg fixed 5 minutes after fertilization. JGG disappeared and the fertilization membrane was formed.



1. MOTOMURA: Materials of the Fertilization Membrane.



I. MOTOMURA: Materials of the Fertilization Membrane.

ON THE CHANGES OCCURRING IN VARIOUS PARTS OF THE
BODY, ESPECIALLY IN THE SPICULES IN ACCORDANCE
WITH THE INCREASE OF BODY LENGTH IN THE
CASE OF THE CALCAREOUS SPONGE,
SYCON OKADAI HÔZAWA

By

NOBUO SASAKI

Biological Institute, Tôhoku Imperial University, Sendai, Japan

(With 12 text-figures)

In classifying the sponges, the spicules they contain form one of the most important characteristics, and thus their shape, length, thickness and the mode of arrangement, etc. are carefully examined. But in general, these features of the spicules seem to be treated as if they were of a constant nature, remaining the same through all the stages of growth.

From the view point above mentioned it is interesting to examine the growth of the spicules in relation to the increase in the length of the sponge body.

The writer has selected as material, a calcareous sponge, *Sycon okadai* HÔZAWA and was able to ascertain some facts concerning the development of the spicules with the increase of the sponge body.

Before preceeding further, the writer wishes here to express his hearty thanks to Professor Dr. SANJI HÔZAWA for his kind directions and helpful advice.

Sycon okadai HÔZAWA was first described by HÔZAWA in the Journal of the Faculty of Science, Imperial University of Tôkyô, Section IV Zoology, Vol. I, Part 5, pp. 302-304, Pl. III, Figs. 18, 19; Text-fig. 10 in 1929.

The type specimen was collected by Dr. YAICHIRÔ OKADA from Misaki in Sôsyû, Kanagawa Prefecture and the specific name was dedicated to him.

As above mentioned the locality where the species was first discovered is Misaki, but afterwards, it was found abundantly in Mangoku-ura, Onagawa-wan and Kesenuma-wan, in Miyagi Prefecture. On the 22nd of May in 1940 Mr. SENJI TANITA collected at Mangoku-ura a great

number of large and small specimens of the same species, growing attached to a small mass of sea weed. The specimens used in this investigation are those just mentioned.

I should like here to mention the general outline of some features of an adult specimen.

The body of the sponge is cylindrical in form. The canal system is of the syconoid type and the flagellated chambers are arranged radially around the central gastral cavity. Each of the flagellated chambers is an elongated sack in form, with its end not branched. The exhalant canal which connects the flagellated chamber with the gastral cavity is rather short. The skeleton of the flagellated chamber which was taken

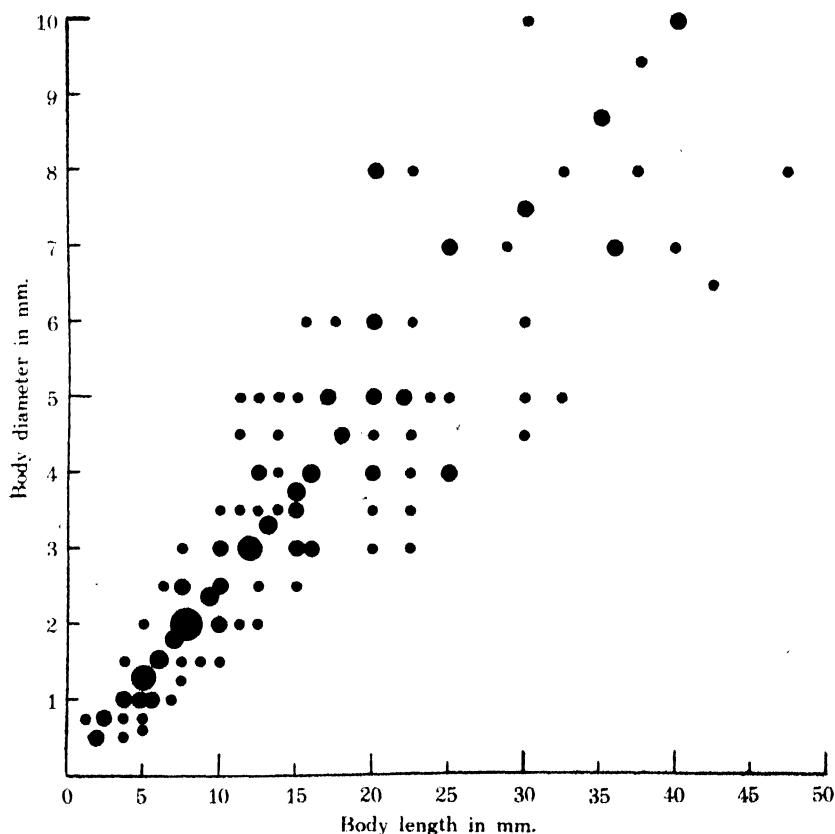


Fig. 1. Graph showing the relation between the body length and the body diameter obtained by measuring one hundred and fifty examples of *Sycon okadae* HÔZAWA.

from the middle portion of the body is composed of triradiates arranged in several joints and of the basal rays of subgastral triradiates situated at the inner end of the former kind of spicules forming the said skeleton. There occurs a tuft of oxea at the distal end of each flagellated chamber. The gastral skeleton forming the wall of the gastral cavity is composed mainly of triradiates and of gastral quadriradiates.

Besides these spicules the paired rays of subgastral triradiates are also added to the same skeleton. Apical rays of the gastral quadriradiates are projected into the gastral cavity.

At first, one hundred and fifty, of the large and small examples of sponges were arbitrarily taken from the above mentioned specimens obtained at Mangoku-ura.

And then each of these examples was measured as to length and diameter.

From the results thus obtained, it was known that the relation between the body length and the body diameter may be represented with a straight line as shown in figure 1.

And thus it was proved that the body length and the body diameter maintain nearly a constant ratio of growth. Five examples, each of which have the dimensions shown in the following lines, were chosen from among the above mentioned examples, and thus the changes occurring especially in the spicules in accordance with the increase of body length were examined.

Of the five examples the smallest and the largest measured respectively 2 mm. and 40 mm. in body length, while the remaining three were inter-

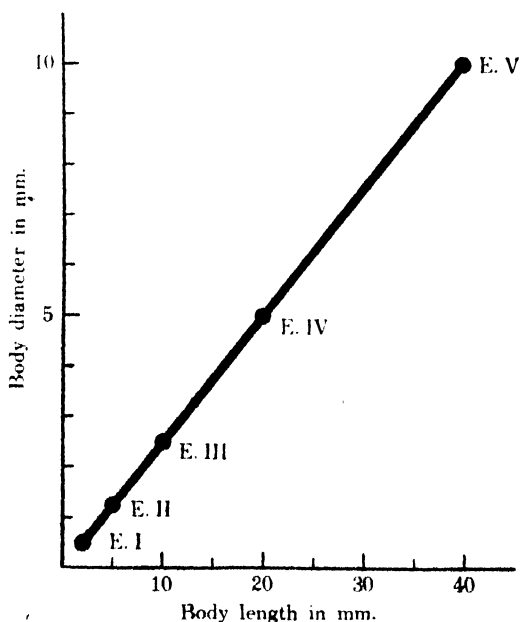


Fig. 2. The curve showing the relation between the body length and the body diameter of five examples from E. I to E. V of *Sycan okadai*.

mediate between them. These five examples made a geometrical progression in both body length and body diameter in nearly an equal ratio of 2 as shown in the following.

The first example called E. I measured 2 mm. in length and 0.5 mm. in diameter. The 2nd example called E. II is 5 mm. long and is 1.2 mm. in diameter.

The 3rd example is 10 mm. long and is 2.5 mm. in diameter and is called E. III.

The 4th example measured 20 mm. in length and 5 mm. in diameter and is called E. IV. The 5th example measured 40 mm. long and 10 mm. in diameter and is called E. V.

From each of the examples above mentioned, five cross-sections were prepared from the middle part of the body, using the celloidin section method, and of each, the arrangement of the skeleton, length and diameter of flagellated chambers, and the diameter of the gastral cavity, were examined.

On the other hand, a small piece of sample was taken from the middle portion of each of the examples and was boiled to make microscopic preparations of spicules in order to observe the form and size of the spicules.

The results thus far obtained are as follows.

Body length and body diameter. — As stated above the body length and body diameter grow maintaining nearly a constant ratio, and it is about 4:1 as shown in figure 2.

Flagellated chambers (Figs. 3, 4). — The flagellated chambers are not yet formed in E. I and first appear in E. II. Of E. II, E. III, E. IV, E. V the length of the flagellated chambers was measured and was shown to be 0.15 mm., 0.75 mm., 1.5 mm., and 2 mm. respectively. Their relative growth ratios were shown by the values of about 5, 2, 1.33.

The diameter of each flagellated chamber was similarly measured, and of E. II, E. III, E. IV, and E. V it was 0.07 mm., 0.15 mm., 0.3 mm., respectively.

The relative growth ratios were shown by the same value of about 2.

From those results, it is known of the flagellated chamber that the growth rate of the length is comparatively great in the early stage and it is much greater than that of the diameter, but it decreases gradually in accordance with the increase of the body length, and after E. IV, the growth rate of the length becomes less than that of the diameter. It is represented with the curve shown in figure 3.

The relation between the flagellated chambers and the body length is shown in figure 1.

The growth ratio of the length of the flagellated chambers, as above stated, is great at first and then decreases gradually, and after E. IV it becomes less than the growth ratio of body length, which may be shown by nearly a constant value of 2. This fact is indicated by a curve (L) shown in figure 4. While, the growth ratios of the diameter of the flagellated chambers of the examples from E. II to E. V when observed, show an equal value of about 2. And thus they are equal to the growth ratio of body length and are proportional to each other. So the relation between the diameter of flagellated chambers and body length may be indicated by a straight line (D) as shown in figure 4.

Gastral cavity. — Next it was considered in what manner the diameter of the gastral cavity is increased. In the case of this kind of sponge the following formula may be presumed.

$$\text{Body diameter} = \text{diameter of the gastral cavity} + 2 \times \text{length of the flagellated chamber}$$

Therefore, the diameter of the gastral cavity and the length of the flagellated chamber are seen to grow in inverse ratio.

Of the examples from E. II to E. V, the diameters of the gastral cavity were found to be 0.7 mm., 1 mm., 2 mm., and 6 mm. respectively and their growth ratios which may be shown by the values of about 1.43, 2, and 3, are gradually increased with the increase of the body

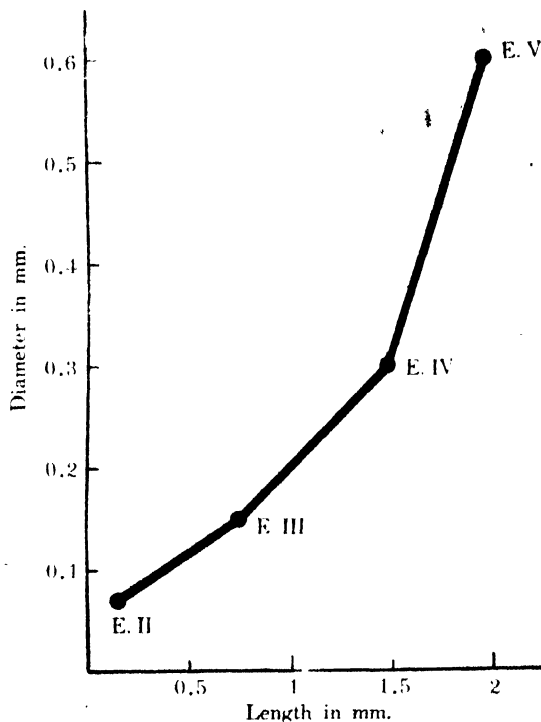


Fig. 3. The growth-curve of the flagellated chambers of four examples, from E. II to E. V of *Sycon okadai*.

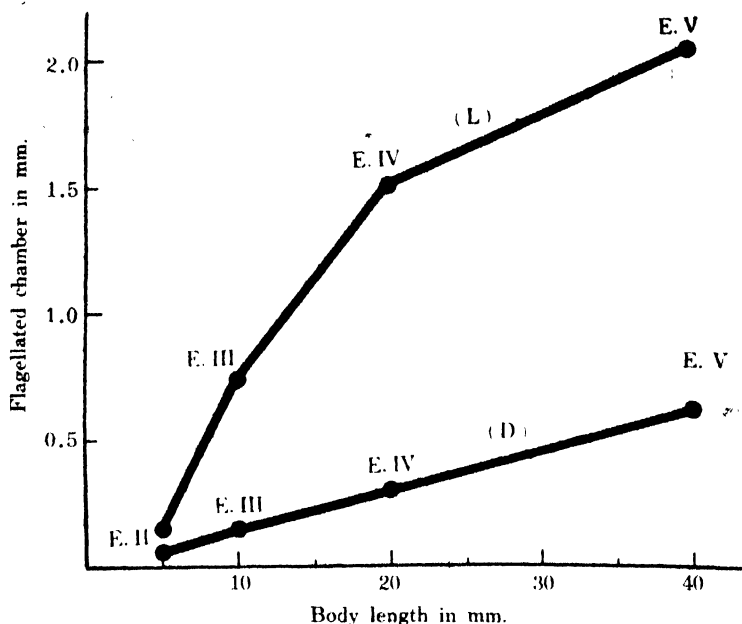


Fig. 4. The growth-relations between the body length and the flagellated chamber of four examples from E. II to E. V of *Sycon okadai*. The curve (L), showing the relation between the body length and the length of the flagellated chamber; the curve (D), showing the relation between the body length and the diameter of the flagellated chamber.

length. Namely, in accordance with the increase of the body length, as above mentioned, the values of growth ratio shown by the flagellated chambers are gradually decreased, while on the contrary, the values of growth ratio shown by the gastral cavity are gradually increased.

These facts may be clearly demonstrated by the actual measurement.

From the results above mentioned, in *Sycon okadai*, it is clear that, when the length of the flagellated chambers gradually diminishes its growth rate, the diameter of the gastral cavity increases its growth rate, compensating the diminution in the growth rate of the length of the flagellated chamber.

Moreover, from the fact that the growth rate of the diameter of the flagellated chamber is exactly equal to that of the body length, it may be concluded that the growth of the body may be accomplished by the increase of the diameters of the flagellated chambers but not by increasing the total number of the same.

TABLE I. Number of flagellated chambers reckoned on the plane of cross-section taken from the middle portion of body of each example.

Example	E. II	E. III	E. IV	E. V
Number of flagellated chambers	27	26	31	30
	38	32	22	35
	40	37	45	29
	32	36	32	38
	30	27	26	40
Average	33.4	31.6	31.2	34.4

As shown in table I, the number of flagellated chambers are reckoned on the plane of the cross-section taken from the middle portion of the body of each example. Judging from these data, though they may be small in number, it is clear that the larger examples do not always have a larger number of flagellated chambers than the smaller, and vice versa. Thus it need not be concluded that the number of flagellated chambers increases in accordance with the growth of the body, but it is more natural to consider that the number of flagellated chambers are nearly

TABLE II. Frequency-table of the length of oxea. The length measured of one hundred oxea taken from each of five examples.

Class	Frequency				
	E. I	E. II	E. III	E. IV	E. V
100-199 μ	18				
200-299 μ	62	40	15	6	4
300-399 μ	20	37	26	5	4
400-499 μ		19	36	21	19
500-599 μ		4	18	40	20
600-699 μ			5	21	23
700-799 μ				6	13
800-899 μ				1	11
900-999 μ					4
1000 μ -					
Total	100	100	100	100	100

constant through all the stages of growth. This fact endorses the conclusion above mentioned.

Spicules. — As above stated, there are several sorts of spicules forming

TABLE III. Arithmetical means and respective standard errors calculated of the length of one hundred oxea taken from each of five examples.

	Length of oxea	Mean	Standard error
E. I	120- 350 μ	244.1 $\mu \pm$	5.44 μ
E. II	200- 550 μ	334.8 $\mu \pm$	6.74 μ
E. III	200- 620 μ	413.1 $\mu \pm$	10.74 μ
E. IV	200- 800 μ	529.7 $\mu \pm$	11.79 μ
E. V	200-1020 μ	606.6 $\mu \pm$	17.41 μ

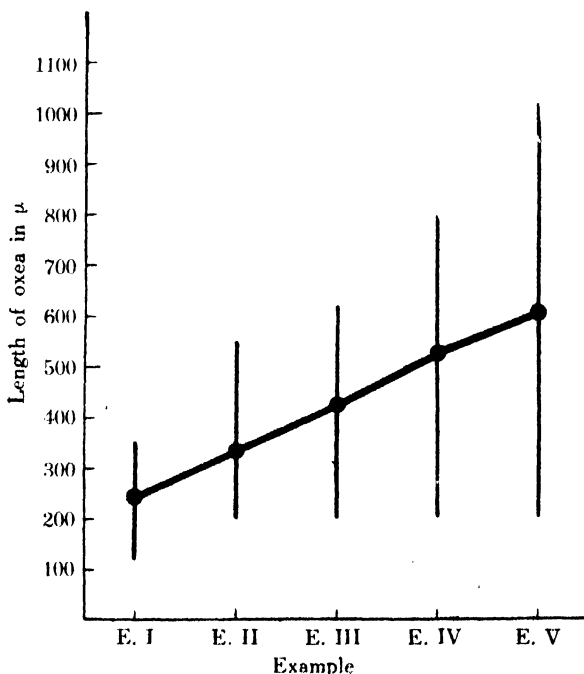


Fig. 5. The growth-curve of the length of five examples from E. I to E. V of *Sycon okadai*. Spots showing arithmetical mean, and vertical lines showing range of variation of one hundred oxea of each five examples which are indicated in table III.

the skeleton of this species, but here only three kinds of spicules, viz. oxea which are seen at the outer end of flagellated chambers, the subgastral triradiates and the gastral quadri-radiates, were selected to investigate.

Oxea (Figs. 5, 6, 7). — The length of one hundred oxea taken from each of five examples from E. I to E. V as shown in the table II was measured, and their respective arithmetical means together with their standard errors were also calculated.

From the results thus far obtained it is clear that the length of oxea grow on a fairly large scale in accordance with the increase of the body length.

That is to say, the growth ratios of the length of this kind of spicules are

1.37, 1.23, 1.28 and 1.15 of each of five examples from E. I to E. V, being calculated from each arithmetical mean.

As is clear from these growth ratios, and from the table III and figure 5, the length of oxea in these examples from E. I to E. V shows equal growth ratios, only representing a slight depression of the same after E. IV. The relation between body length and the length of oxea may be manifested by a curve as shown in figure 6.

The growth rate of body length is

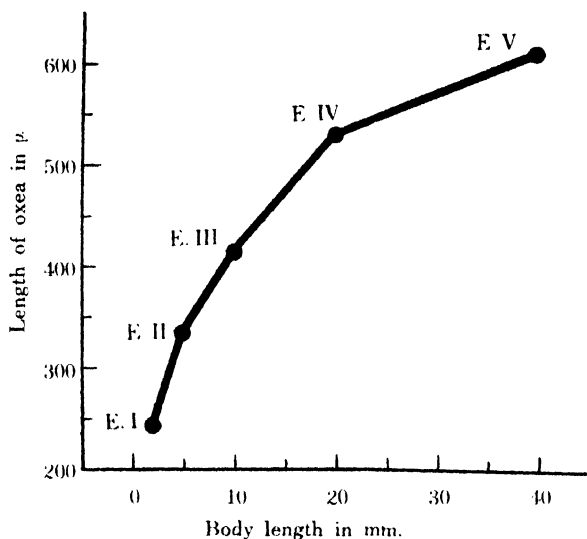


Fig. 6. The curve showing the relation between the body length and the length of oxea of each of five examples of *Sycon okadai*.

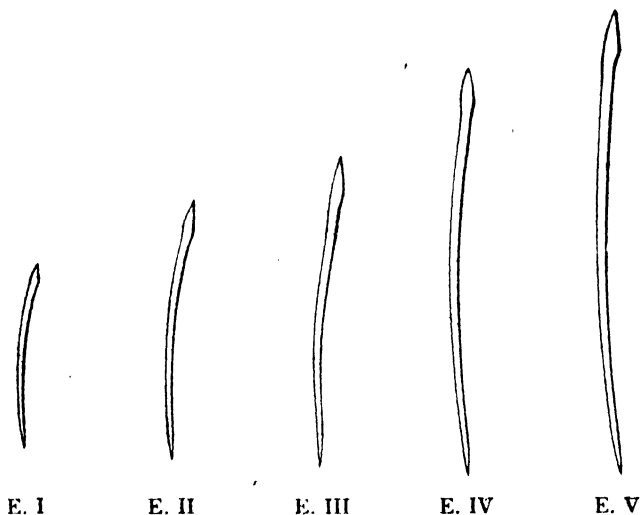


Fig. 7. The oxea of *Sycon okadai*, illustrated by the respective mean value of five examples which are shown in table III. ($\times 100$).

greater than that of the length of oxea.

Therefore the body length and the oxea do not grow in the same proportion and thus when the body length is more increased the difference existing between them will become more distinct. Consequently, the smaller specimen has comparatively larger oxea, and on the contrary, the larger specimen has smaller oxea. The oxea which show the respective mean value of the said five examples are illustrated in figure 7.

Subgastral triradiates (Figs. 8, 9, 10). — The length of basal ray and paired rays of fifty examples of the subgastral triradiates were measured, and their arithmetical means and standard errors were also calculated as in the case of the oxea. But in E. I, on account of the fact that the flagellated chambers are not yet perfectly formed, the number of this kind of spicules is very scarce, and thus only ten spicules were calculated. The results thus obtained are shown in table IV and table V.

From these tables it is clear that both the basal ray and paired rays of subgastral triradiates grow in accordance with the increase of the body length.

In the next, the mode of growth of this kind of spicule will be examined more precisely.

TABLE IV. Frequency-table of the length of basal rays and paired rays of subgastral triradiates. The length of basal rays and paired rays of ten subgastral triradiates taken from example E. I were measured, and of fifty of this kind of spicules taken from each of the remaining four examples.

Class	Basal rays				
	Frequency				
	E. I	E. II	E. III	E. IV	E. V
50–79 μ	10	9			
80–109 μ		21	1		
110–139 μ		14	1	1	
140–169 μ		6	8	6	5
170–199 μ			18	10	3
200–229 μ			17	19	21
230–259 μ			5	11	16
260 μ				3	5
Total	10	50	50	50	50

Class	Paired rays				
	Frequency				
	E. I	E. II	E. III	E. IV	E. V
30- 49 μ	10	11			
50- 79 μ		31	3		
80-109 μ		8	18	8	2
110-139 μ			26	17	13
140-169 μ			3	21	11
170-199 μ				4	19
200 μ -					5
Total	10	50	50	50	50

TABLE V. Arithmetical means and respective standard errors of the length of basal ray and paired rays of ten subgastral triradiates taken from example E. I were calculated, and of fifty of this kind of spicules taken from each of the remaining four examples.

	Basal rays			Paired rays		
	Length of the rays	Mean	Standard error	Length of the rays	Mean	Standard error
E. I	55- 78 μ	65.3 μ	$\pm 2.64 \mu$	30- 40 μ	34.7 μ	$\pm 0.90 \mu$
E. II	62-155 μ	104.1 μ	$\pm 3.89 \mu$	38- 95 μ	59.8 μ	$\pm 2.23 \mu$
E. III	100-240 μ	191.6 μ	$\pm 4.24 \mu$	50-140 μ	107.6 μ	$\pm 2.68 \mu$
E. IV	120-280 μ	204.8 μ	$\pm 4.91 \mu$	80-180 μ	133.2 μ	$\pm 3.39 \mu$
E. V	140-280 μ	218.4 μ	$\pm 4.30 \mu$	100-210 μ	157.6 μ	$\pm 4.37 \mu$

The basal rays of five examples from E. I to E. V were calculated, and their growth ratios were obtained from respective arithmetical means.

The value of the growth ratio thus far obtained was 1.59, 1.84, 1.07 and 1.07 respectively.

From these ratios, and from the table V and the curve represented with full-line (B. R.) in figure 8, it is clear that the growth rate of the basal ray of subgastral triradiates is the highest between E. II and E. III, and is comparatively high between E. I and E. II. But after E. III it diminishes remarkably. That is to say, the growth of the basal ray of the subgastral triradiate is mainly performed during the period covering E. I to E. III and is most vigorous at the stage between E. II and E. III.

After E. III its growth becomes very slow.

The growth ratios of the paired rays of the subgastral triradiate were also calculated, in the same manner as before, from the means of five examples taken from E. I to E. V, and each was shown respectively by the value of 1.76, 1.80, 1.24 and 1.18.

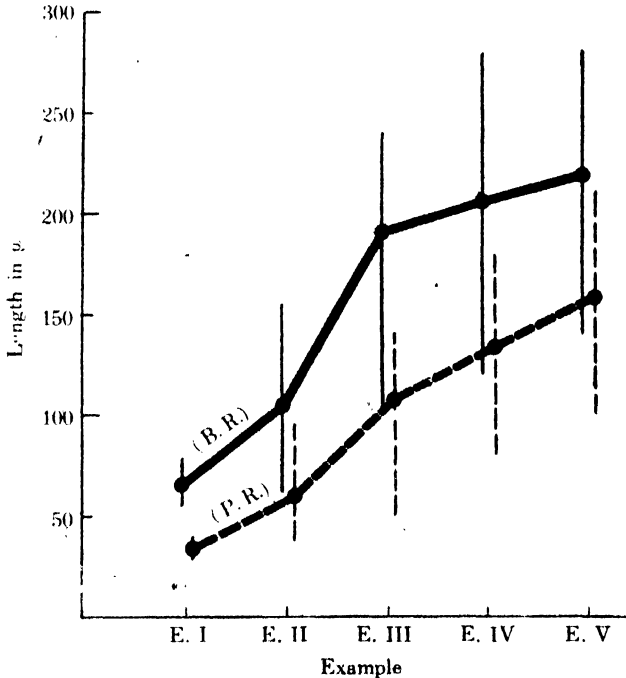


Fig. 8. The growth-curve of the length of basal rays and paired rays of subgastral triradiates of each five examples of *Sycon okadai* which are shown in table V. The spots expressing arithmetical mean and the vertical lines showing the range of variation. The curve in full-line (B. R.) shows growth-curve of the basal ray and the curve in broken-line (P. R.) shows growth-curve of the paired rays.

Judging from these values, and from table V and the curve mentioned with broken-line (P. R.) in figure 8, the growth rate of the paired rays of the subgastral triradiate seems to be highest between E. II and E. III and is high between E. I and E. II and after E. III, it gradually decreases. Thus the growth of the paired rays of the subgastral triradiate is mainly performed during the stages from E. I to E. III, as in the case of the basal ray. But the paired rays will continue to grow maintaining a high growth rate even after they pass E. III.

The growth relation existing between the basal ray and the paired rays was represented with a curve shown with full-line (S. T.) in figure 9.

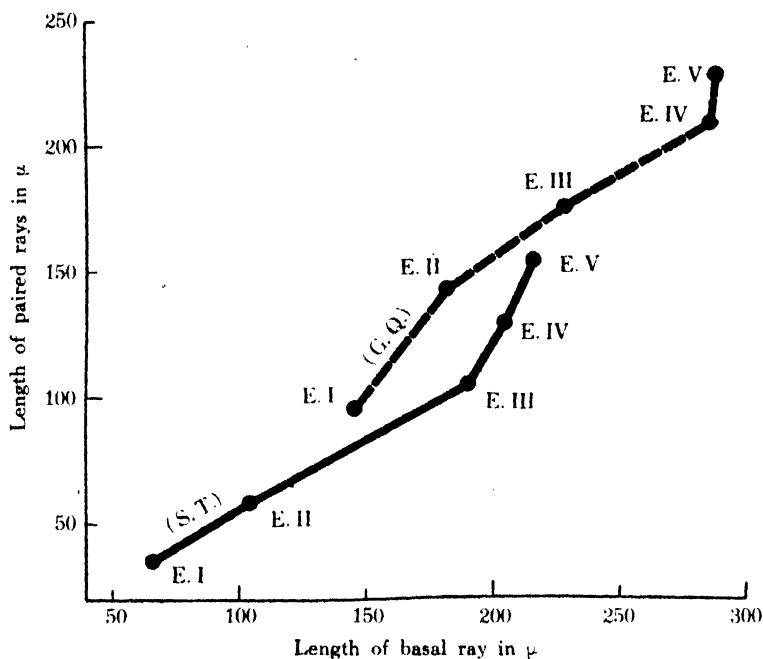


Fig. 9. Growth-relations between the basal ray and the paired rays of subgastral triradiates and those of gastral quadriradiates of each of five examples of *Sycon okadai*. The curve in full-line (S. T.) indicates the growth of subgastral triradiates and the curve in broken-line (G. Q.) expresses growth of gastral quadriradiates.

Here a sudden change in growth rate can be recognized at a point in E. III.

That is to say, the growth rate of the basal ray of the subgastral triradiates is larger than that of the paired rays at the stages from E. I to E. III, while on the contrary, the growth rate of paired rays is larger than that of the basal ray at the stages from E. III to E. V.

The subgastral triradiates which show the respective mean value of the above mentioned five examples are illustrated in figure 10.

Gastral quadriradiates (Figs. 9, 11, 12). — The basal ray and the paired rays of fifty examples of gastral quadriradiates were measured, and their arithmetical means and standard errors were also calculated.

The results thus far obtained are shown in tables VI and VII.

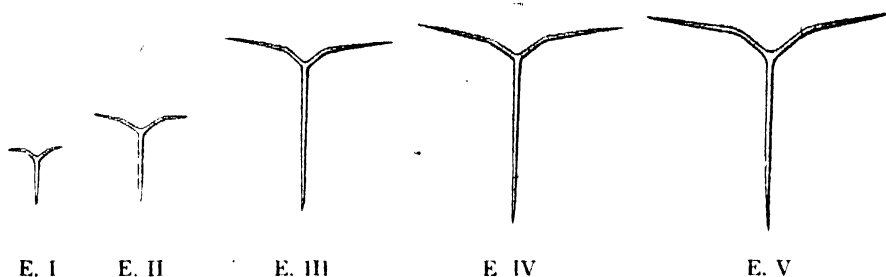


Fig. 10. The subgastral triradiates of *Sycon okadai*, illustrated by the respective mean value of five examples shown in table V.

TABLE VI. Frequency-table of the length of basal rays and paired rays of gastral quadriradiates. The length of basal rays and paired rays of fifty gastral quadriradiates taken from each of five examples was measured.

Basal rays					
Class	Frequency				
	E. I	E. II	E. III	E. IV	E. V
50-99 μ	2				
100-149 μ	16	7			
150-199 μ	32	21	9		
200-249 μ		21	26	8	9
250-299 μ		1	10	15	17
300-349 μ			4	24	17
350-399 μ			0	1	5
400 μ -			1	2	2
Total	50	50	50	50	50

Paired rays					
Class	Frequency				
	E. I	E. II	E. III	E. IV	E. V
50-99 μ	22	1			
100-149 μ	28	24	6		
150-199 μ		25	30	15	5
200-249 μ			14	27	30
250-299 μ				8	13
300 μ -					2
Total	50	50	50	50	50

TABLE VII. Arithmetical means and respective standard errors of the length of basal rays and paired rays of gastral quadriradiates taken from each of five examples were calculated.

	Basal rays			Paired rays		
	Length of the rays	Mean	Standard error	Length of the rays	Mean	Standard error
E. I	80-180 μ	146.1 μ	$\pm 3.14 \mu$	50-130 μ	96.5 μ	$\pm 2.86 \mu$
E. II	100-250 μ	183.4 μ	$\pm 4.77 \mu$	90-190 μ	144.2 μ	$\pm 3.05 \mu$
E. III	150-400 μ	228.6 μ	$\pm 7.24 \mu$	120-240 μ	177.6 μ	$\pm 3.82 \mu$
E. IV	200-420 μ	287.2 μ	$\pm 6.19 \mu$	150-270 μ	210.8 μ	$\pm 4.23 \mu$
E. V	200-430 μ	289.2 μ	$\pm 6.95 \mu$	160-330 μ	230.4 μ	$\pm 4.35 \mu$

Judging from these results it is clear that both the basal ray and the paired rays of this kind of spicule will grow in accordance with the increase of the body length. In the following lines the mode of growth of the said rays of spicules will be discussed more precisely.

The growth ratios were calculated by the arithmetical means of basal ray of gastral quadriradiates and of five examples taken from E. I to E. V. They were shown by such values as 1.26, 1.25, 1.26, and 1.01.

From these ratios, from the table VII

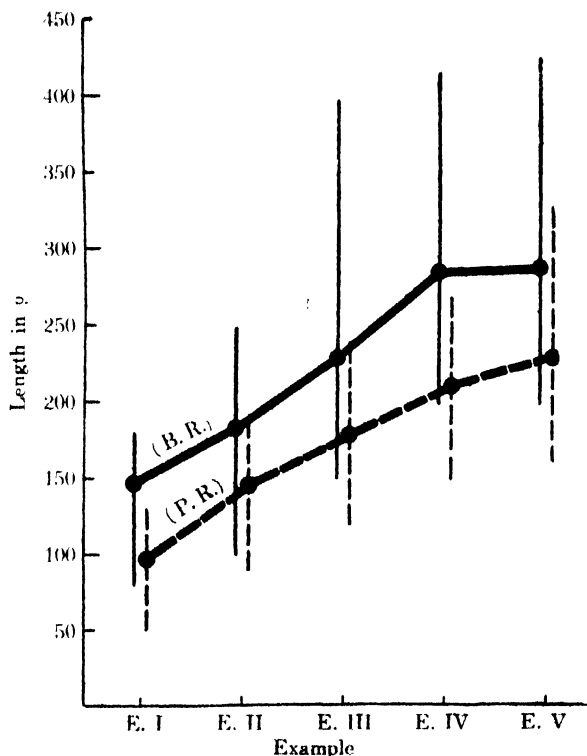


Fig. 11. The growth-curves of the length of basal rays and paired rays of the gastral quadriradiates of each of five example of *Sycon okadae*, which are shown in table VII. The spots expressing arithmetical mean and the vertical line expressing the range of variation. The curve in full-line (B.R.) shows growth-curve of the basal rays and the curve in broken-line (P.R.) indicates growth-curve of the paired rays.

and the curve mentioned with a full-line (B. R.) in figure 11, it is clear that the growth rate of the basal ray of gastral quadriradiate shows nearly an equal value in the cases of examples taken from E. I to E. IV and is suddenly diminished at the stage standing between IV and E. V. Thus the basal rays in the stages from E. I to E. IV, grow actively showing nearly an equal ratio, while the same grow very slowly after the stage E. IV.

The growth ratios of the paired rays of the same kind of spicule were also calculated of five examples of from E. I to E. V, obtaining the arithmetical means.

The growth ratios were 1.49, 1.23, 1.19 and 1.09.

From these ratios, and from table VII and a curve mentioned with a broken-line (P. R.) in figure 11, it seems to be clear that the paired rays of the gastral quadriradiate show at first, a comparatively large then a gradually decreasing growth rate when observed through the stages from E. I to E. V. That is to say, the paired rays of the gastral quadriradiate of the examples from E. I to E. V grow continuously, though their growth rate is gradually diminished.

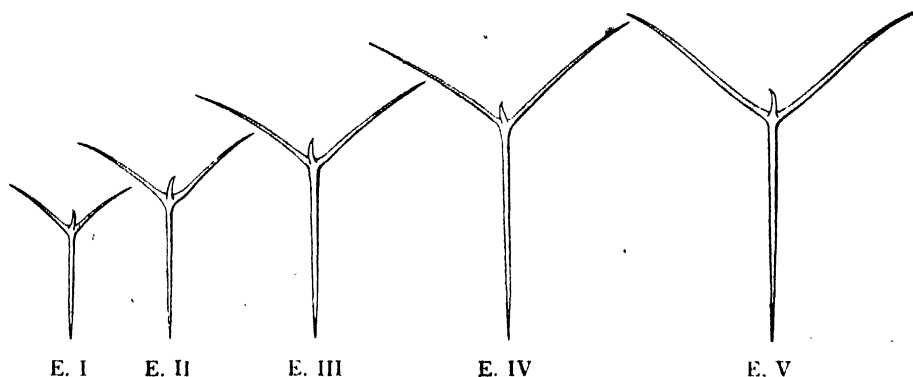


Fig. 12. The gastral quadriradiates of *Sycon okadai*, illustrated by the respective mean value of five examples which are shown in table VII.

The relation between the basal ray and the paired rays of the gastral quadriradiate, was shown by the curve mentioned with broken-line (G. Q.) in figure 9. As is seen from the curve, at the stages from E. I to E. II, the paired rays grow in a rate slightly greater than that of the basal ray, while at the stages from E. II to E. IV, the basal ray grows at a rate greater than that of the paired rays. After passing the stage E. IV,

the growth rate of the basal ray becomes very small, while the paired rays continue to grow. Therefore the curve changes its direction suddenly at a point situated in E. IV. The gastral quadriradiates which show the respective mean value of the five examples are illustrated in figure 12.

Subgastral triradiates and gastral quadriradiates (Figs. 8, 9, 11).— Here the mode of growth of both of the subgastral triradiate and gastral quadriradiate was compared. From figure 8, figure 9, and figure 11, it is clear that the growth rate of the subgastral triradiate is generally higher than that of the gastral quadriradiate. The growth rates of the basal ray and paired rays of both kinds of spicules, will decrease in accordance with the increase of body length, and the decrease in the growth rate occurs in the basal ray earlier than in the paired rays. The sudden diminution of the growth rate in the basal rays takes place at a different time in both kinds of spicules. Namely, in the subgastral triradiate it happens at the stage of E. III, while in the gastral quadriradiate it appears later and in the stage of E. IV. That is to say, the time in which the sudden depression of growth rate appears is different in the cases of subgastral triradiate and gastral quadriradiate. In the former, it comes earlier than in the latter.

SUMMARY

1. In *Sycon okadai* HÓZAWA, the body diameter, the flagellated chambers and the gastral cavity grow in accordance with the increase of body length. And the calcareous spicules also will continue to grow until the former stop their growth.

2. The flagellated chambers are not yet formed when the specimens are less than 2 mm. in body length, but are seen in the specimen of 5 mm. body length.

3. The growth rate of the length of the flagellated chamber is much greater than that of the diameter while the sponge is small, but it is gradually decreased in accordance with the increase of body length, and at last it becomes smaller than that of the diameter.

4. The growth of the diameter of the gastral cavity appears gradually in accordance with the increase of body length. Therefore, the decrease of the growth rate in the length of the flagellated chambers is moderately compensated by the increase of the growth rate of the diameter of the gastral cavity.

5. Judging from the facts that the growth rate of the diameter of

the flagellated chamber is equal to that of the body length and that the number of the flagellated chambers seems to be nearly constant, not withstanding the size of the sponge, the growth of the sponge body is accomplished by the increase of the diameter of the flagellated chambers but not by the increase of the total number of flagellated chambers after the sponge has attained some length of body.

6. Though the mode of growth of each kind of spicule may be different, the growth rate of spicules is more remarkably decreased in the later stages than in the early. As the growth rates of the spicules are lower than that of the body length, the smaller specimen has comparatively large spicules and the larger one has comparatively smaller spicules.

7. The oxea grow with nearly an equal growth rate in the cases of the specimens from 2 mm. to 40 mm. body length. But a considerable diminution of growth rate is seen in the case of the spicules of later stages.

8. The basal rays and the paired rays of the subgastral triradiates when observed of the specimens from 2 mm. to 10 mm. body length, grow vigorously and show a high growth rate, while the growth rate of the specimens from 10 mm. to 40 mm. body length becomes very slow, showing a conspicuous decrease. Especially the diminution of the growth rate of the basal rays is more conspicuous than of the paired rays, and thus it may be concluded that they stop growing.

9. The basal ray of the gastral quadriradiates will grow with nearly an equal growth ratio in the same specimens from 2 mm. to 20 mm. body length, but later they show a sudden diminution of the same, as if they stopped growing. While the paired rays of the same kind of spicule taken from the specimen of from 2 mm. to 40 mm. body length grow with a large growth ratio. But in this case also the rate shown in the later stage is smaller than that shown in the earlier stage.

10. Generally the growth rate of the subgastral triradiate is greater than that of the gastral quadriradiate. If the basal ray of the subgastral triradiate is compared with the same of the gastral quadriradiate, the former begins to grow very slowly at the stage of 10 mm. body length, while in the case of the latter, the same phenomenon comes at the stage of 20 mm. body length.

ACTINIARIA COLLECTED IN THE VICINITY OF ONAGAWA BAY

By

TOHRU UCHIDA

Zoological Institute, Faculty of Science, Hokkaido Imperial University

(With 5 text-figures)

(Received May 5, 1941)

The collection forming the basis of this report was secured by the authorities of the Onagawa Oceano-chemical Institute of the Tôhoku Imperial University during the summers of 1935 and 1936. All the specimens were preserved in formalin, therefore, they are mostly more or less contracted and their coloration has nearly faded out. These actinian specimens mostly belong to common littoral forms which are widely distributed along the coasts of North Japan, but the single species, *Stomphia coccinea*, which is a common circumboreal one, has been recorded for the first time in Japanese waters. The two species, *Edwardsia japonica* and *Actinostola Carlgreni*, both reported on the basis of a single specimen from Japan, have been again examined in this collection. Before proceeding further, the writer must express his cordial thanks to Prof. S. HÔZAWA and Dr. T. IMAI for undertaking the publication of this report and for specimens placed at the writer's disposal.

LIST OF SPECIES

Tribe *Nynantheae*

Subtribe *Athenaria*

Family *Edwardsiidae*

1) *Edwardsia japonica* CARLGREN

Family *Halcampoidae*

2) *Eloactis mazellii* (JOURDAN)

Subtribe *Endomyaria*

Family *Boloceridae*

3) *Eubolocera multicornis* (VERRILL)

Family *Bunodactiidae*

- 4) *Anthopleura xanthogrammica* BRANDT
- 5) *Anthopleura stella* (VERRILL)
- 6) *Anthopleura japonica* VERRILL
- 7) *Tealia felina* LINNAEUS var.
- 8) *Epiactis prolifera* VERRILL

Subtribe *Acontiaria*Family *Diadumenidae*

- 9) *Diadumene luciae* (VERRILL)

Family *Metridiidae*

- 10) *Metridium senile* var. *fimbriatum* VERRILL

Family *Hormatiidae*

- 11) *Calliactis* sp.

Subtribe *Mesomyaria*Family *Actinostolidae*

- 12) *Actinostola carlgreni* WASSILIEFF
- 13) *Stomphia coccinea* (MÜLLER)

Edwardsia japonica CARLGREN

Edwardsia japonica: CARLGREN, 1931, pp. 12-13.

Column vermiform, divisible into physa, scapus, scapulus and capitulum, 24 mm long and 4 mm in diameter. Physa small but distinct, swollen and probably not retractile into the scapus, with a terminal pore. Scapus has a rusty brown investment which is greyish brown above and may be readily peeled off. Scapus divided into 8 broad longitudinal ridges. Nemathybomes small, scattered on the surface of the scapus. They could not be observed without making sections. Scapulus about 3 mm long and smooth. Capitulum short and smooth as the scapulus. Disc destitute of coloured markings in this preserved specimen, with 8 distinct lobes. Mouth slit-like. Tentacles 20 in number, short, broad and bluntly pointed, with shorter ones inwards.

The mesenteries are 8 and all perfect. They are provided with well

developed retractor muscles which have 25-30 folds in the fertile region. Most of the folds are branched from their bases, especially complicatedly in the outer part of the retractor near the body wall. To the end of the inner edge of the retractor continues the fertile zone which includes many ova and oöcytes. The outer lamellar part of the mesenteries are attached to the retractor at some distance from its outer edge. Parietal muscles in the upper part of the fertile region somewhat triangular in section, with a few broad processes.

Locality. The single specimen was collected from Okati Bay on July 1, 1937.

Remarks. The specimen in general coincides with the description of *Edwardsia japonica* which was given by CARLGREN (1931) based on a single specimen collected at the depth of 2-3 fathoms in Sagami Bay. CARLGREN's specimen which was strongly contracted seems to be younger in stage than the present specimen. The difference of folds of retractors in the two specimens seems to be due to the levels at which they were sectioned.

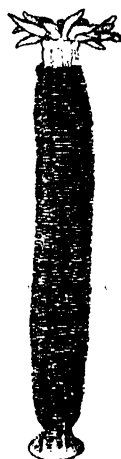


Fig. 1. *Edwardsia japonica* CARLGREN
× 2.

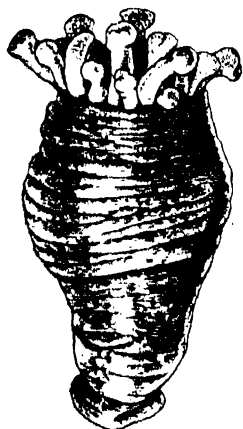


Fig. 2. *Eloactis mazellii*
(JOURDAN) × 2.

Eloactis mazellii (JOURDAN)

Eloactis mazellii: UCHIDA, 1938, pp. 288-289

One specimen was collected off Nakanoshima and three specimens were dredged at Oura on July 15, 1935. This actinian is one of the common Northern forms.

Distribution in Japan. Mutsu Bay, Onagawa Bay.

Eubolocera multicornis (VERRILL)

Eubolocera multicornis: VERRILL, 1922, p. G 117, pl. 23, fig. 2; UCHIDA, 1940, pp. 268-269.

Bolocera brevicornis: McMURRICH, 1893, pp. 159-160, pl. 23, Figs. 31-33.

A single large specimen was collected by a trawl at a depth of 110 fathoms, 12 miles east from Kinkazan on January 2, 1935.

Distribution in Japan. Akkeshi and Onagawa Bay

***Anthopleura xanthogrammica* BRANDT**

Anthopleura xanthogrammica: UCHIDA, 1938, pp. 298-302.

Two specimens were collected on a shingle of Takosima on August 28, 1935. They are typically contracted in preservative. This is the common shore form on the coasts of Japan and the United States of America.

Distribution in Japan. Akkeshi, Muroran, Asamushi and Onagawa Bay.

***Anthopleura stella* (VERRILL)**

Anthopleura stella: UCHIDA, 1938, pp. 293-298.

A single specimen which was obtained in Yatarosima on Aug. 27, 1935 has been identified with this common Japanese species.

Distribution in Japan. From north-western coasts of Hokkaido (such as Oshoro, Hakodate) through Honshu to the southern coasts of Kyushu.

***Anthopleura japonica* VERRILL**

Anthopleura japonica: UCHIDA, 1938, pp. 302-305.

Several specimens collected by Dr. S. KOIZUMI were examined by the writer.

Distribution in Japan. From northern parts of Honshu to southern coasts of Kyushu.

***Tealina felina* (LINNAEUS) var.**

Tealina felina: STEPHENSON, 1937, pp. 139-155.

Two specimens of an actinian may be possibly referable to the species. A specimen collected by a trawl at a depth of 130 *hiro* off Kinkazan on Dec. 28, 1935 is 35 mm high and 45 mm wide in the pedal disc. Another specimen collected by a trawl at a depth of 160 *hiro* off Kinkazan on Jan. 21, 1936 is 85 mm high and 120 mm wide at the base. These specimens are different from *T. felina* var. *coriacea* in the possession of a larger size and the occurrence in deeper waters. Though similar in size and deep habitats to *T. felina* var. *lofotensis*, they are distinguished from the variety in the possession of distinct verrucae which are rather thickly set and more or less arranged in many vertical rows. The verrucae

are very flat and not so prominent as in the description of *T. felina* var. *tuberculata*. Two preserved specimens are conical in shape, having a widened base and a narrowed oral disc. The wall of column is stout and thick. The pedal disc is extended and stout in the preservative, covering the substratum. This form has never previously been found in Japanese waters.

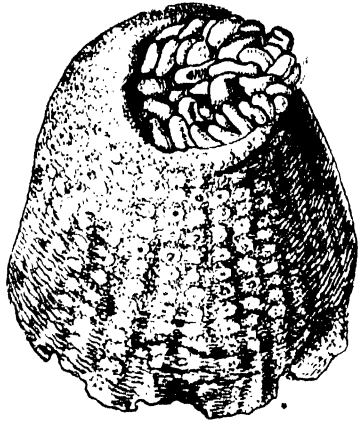


Fig. 3. *Tralia felina* LINNEUS
var. $\times 1$.

Epiactis prolifera VERRILL

Epiactis prolifera. UCHIDA, 1938, pp. 309
310.

A large specimen bearing many embryos was collected by Mr. K. KIMURA in Hirashima on May 8, 1935. Six specimens with conspicuous papillae at the middle level of the column were examined. Amongst them two large ones were observed to bear embryos. They were collected in Kasagaishima on July 16, 1935. This actinian is one of the common North Pacific forms.

Distribution in Japan. Around the coasts of Saghalien and Hokkaido. On the coasts of Honshu from Misaki southward to Mutsu Bay.

Diadumene luciae (VERRILL)

Diadumene Luciae: UCHIDA, 1938, pp. 313-314.

A single large specimen was obtained in Koshiki on July 18, 1935 and about 20 small ones on the coast on Niiko on Aug. 19, 1935.

Distribution in Japan. From Hokkaido to the Loo Choo Islands, very common.

Metridium senile var. *fimbriatum* VERRILL

Metridium senile var. *fimbriatum*: UCHIDA, 1938, pp. 314-315.

Two large specimens were collected by Mr. K. KIMURA on the coast of Hirashima on May 8, 1935 and two specimens of moderate size were caught in Izusima on July 10, 1937. The locality is the most southwestern point where they have been found in Japan.

Distribution in Japan. Around the coasts of the Kurile Islands and

Hokkaido. In Honshu, Mutsu Bay and Onagawa Bay.

Calliactis sp.

Two actinians belonging to the genus were found in Koyatori Bay on July 18, 1935. They were firmly attached to a Gastropod shell as they were not in good preserved condition, the specific identification cannot be given at the present time.

Actinostola carlgreni WASSILIEFF

Actinostola carlgreni: WASSILIEFF, 1908, pp. 28-31.

An example slightly contracted and narrowed aborally, 65 mm high and 50 mm wide in the oral region and 30 mm wide in the pedal disc.

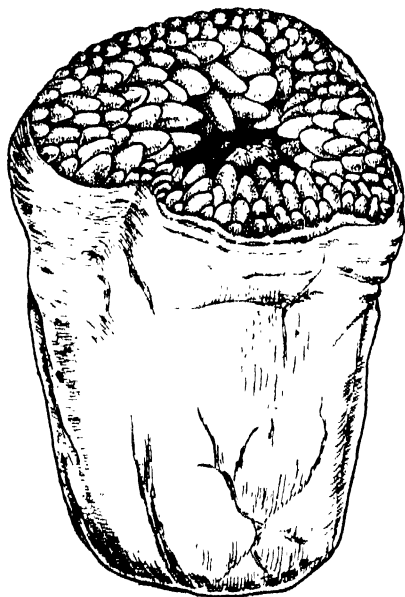


Fig. 4. *Actinostola carlgreni* WASSILIEFF
<1.

Oral disc partly concealed by a large number of short, broad tentacles which are not strongly contracted. Column nearly cylindrical and slightly smaller above than below. Its wall is smooth and has no indication of verrucae. Mesogloea thick and robust. Pedal disc contracted inwards and possessing foldings set radially. Pedal disc and oral disc furnished with a thickened ectoderm and tinted slightly brownish in the preserved specimen. Column white and parchment-like. Tentacles arranged about in seven cycles, with the larger ones inwards. They are short and broad, and the terminal pit is observable. The mesogloea of them contains many bundles of longitudinal muscles. In sections

of the upper half the bundles universally surround the endodermal canal, while in sections near the base they are mostly restricted to the outer side. The sphincter on the discal margin is well developed and is represented by a large number of muscular bundles, which are thickly distributed nearly the whole width of mesogloea near the margin, but

are limited to the region near the endoderm in the lower part. The sphincter extends $\frac{2}{3}$ the length of the column. As it becomes faint toward the aboral end, the endodermal muscles corresponding to it becomes strong. Mesenteries are arranged in five cycles, 6, 6, 12, 24, 48. The first and the second ones are perfect. Out of the first cycle, two pairs are directives. The mesenteries of the first four cycles are fertile, while those of the fifth cycle are sterile and not always complete in number in all divisions. The longitudinal muscles of the mesenteries are not well developed which is the usual case in the genus. The longitudinal muscles of these mesenteries are best developed in the mesenteries of the first series, and next in those of the second series. In these mesenteries the muscles are limited to the inner margin in the directives to the outer margin and slightly folded. In the third and fourth mesenteries the folds are still more indistinct and in the fifth they are obscure. Stomodaeum, reaching further down half the length of the column, folded. A single specimen was examined. The locality is unlabelled.

Distribution in Japan. Sagami Bay and Onagawa Bay.

Stomphia coccinea (MÜLLER)

Stomphia coccinea: McMURRICH, 1911, pp. 77-79; STEPHENSON, 1935, pp. 381-389; PAX, 1936, p. c 102.

Stomphia carnea: VERRILL, 1922, pp. 118 G-119 G.

Three small specimens were collected in Oura by trawl at the depth of 160 *hiro* off Kinkazan on Dec. 28, 1935 and another one was found by a trawl at the depth of 150 *hiro* off Ozaki on March 28, 1936. This is the first record from Japanese waters. The actinian is one of the circumboreal ones and is known as an abyssal form. Out of the five specimens examined by the writer three are young and two are comparatively large, measuring about 30 mm wide and 20 mm wide and having well-developed gonads. These preserved specimens are variable in shape, columnar or low-dome-like. Tentacles all contracted. The arrangement of them is said

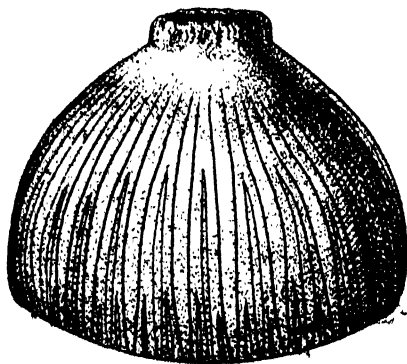


Fig. 5. *Stomphia coccinea* (MÜLLER) $\times 2$.

to be peculiar as follows: 6, 10, 16, 32. They are rather short, stout, tapering to blunt points. Column wall thin and translucent, revealing the insertions of mesenteries. Base wide, flat, adherent, and thin, showing the merenterial insertions. Coloration nearly faded out but retaining slightly pinkish hue.

Distribution in Japan. Onagawa Bay. This actinian probably occurs in the abyss along the Pacific coasts of Northern Japan.

LITERATURE CITED

1. CARLGREN, O. 1931. Zur Kenntnis der Actinaria Abasilaria. Arkiv f. Zool., Bd. 23 A, pp. 1-48.
2. McMURRICH, J. P. 1893. Report on the Actiniae collected by the United States Fish Commission Steamer Albatross during the Winter of 1886-1887. Proc. U. S. Nat. Mus., Vol. 16, pp. 119-216.
3. - . 1911. The Actinaria of Passamaquoddy Bay, with a discussion of their Synonymy. Trans. Roy. Soc. Canada, Ser. 3, Vol. 4, pp. 59-83.
4. STEPHENSON, T. A. 1935. The British Sea Anemones, Vol. 2, pp. 1-426.
5. UCHIDA, T. 1938. Report of the Biological Survey of Mutsu Bay. 33. Actinaria of Mutsu Bay. Sci. Rep. Tohoku Imp. Univ. Ser. 4, Biology, Vol. 13, pp. 281-317.
6. - . 1940. The Fauna of Akkeshi Bay. X. Actinaria. Jour. Fac. Sci. Hokkaido Imp. Univ., Ser. 6, Zoology, Vol. 7, pp. 265-275.
7. WASSILIEFF, A. 1908. Japanische Actinien. Beitr. z. Naturw. Ostasiens, 1. Suppl.-Bd, 2. Abhandlg., pp. 1-49.

ON SOME EARTHWORMS FROM THE SOUTH SEA ISLANDS. II.¹⁾

By

SHINJIRO KOBAYASHI

Keijō Second Higher Common School

(With 4 text-figures)

(Received May 23, 1941)

Through the kindness of Professor TEISŌ ESAKI, the writer has again had the opportunity of examining the earthworms which were collected by him, and also by Messrs. K. YASUMATSU and S. YOSHIMURA, in the South Sea Islands. They belong to the following seven species: *Drawida barwelli typica*, *Dr. sp.*, *Pheretima taitensis*, *Ph. padasensis*, *Ph. sangirensis*, *Dichogaster bolau*i and *Pontodrilus sp.* Of these, the occurrence of *Ph. padasensis* and *Ph. sangirensis* is the first record from the Micronesia. The second form which was mentioned above as *Drawida sp.* appears to be specifically distinct from *Dr. barwelli*, and the last form *Pontodrilus sp.* seems to be a new one, but the writer was unable to identify the species definitely as the specimens of both forms had been extremely macerated and are yet immature.

The writer wishes to express his sincere thanks to Prof. T. ESAKI, and to Messrs. K. YASUMATSU and S. YOSHIMURA, for the kindness given to the writer in supplying the materials. The writer is also greatly indebted to Prof. SANJI HŌZAWA for his continual guidance given during the course of his study.

1. *Drawida barwelli* (BEDDARD) *typica* 1886

(Fig. 1)

1923 *Drawida barwelli typica*, STEPHENSON, Fauna Br. India, Oligochaeta, p. 133.

Description:

External characteristics: The posterior part of the body is incomplete, but the lost part appears to be small. Body-length 22 mm+, greatest diameter 2.8 mm, number of segments 82+. The body, excepting the head region, is somewhat flattened dorsoventrally. Colour, dorsally greenish

¹⁾ Results of Professor T. ESAKI's Micronesia Scientific Expedition 1936-1940, No. 40.

to yellowish brown, ventral surface lighter than the dorsal. Clitellum dark green (the glandularity is not yet completely developed). Prostomium prolobous. Dorsal pores ill-defined, being found behind the clitellar region; but, as the specimen is strongly contracted, the writer was unable to discover the accurate position of the first dorsal pore and also was unable to know whether they are functional or not. Clitellum in X-XIII; the ventral part is not glandulated (this may possibly be attributed to the immaturity of the specimen).

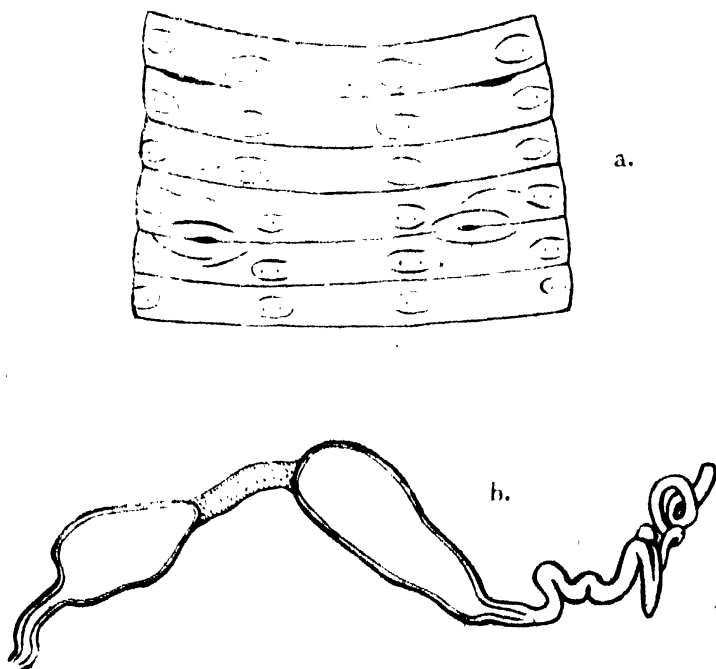


Fig. 1. *Drawida barwelli* typical. a, ventral view of VII-XII, \times ca. 18; b, spermathecae, \times ca. 42.

Setae delicate, closely paired; both ventral and lateral ones present on II, but poorly developed. Setae on the genital region and on the segments behind, about XXII are planted on the inconspicuous tumescences (artificially produced?). Seta distance ab subequal to cd , dd longer than $\frac{1}{2}$ of the circumference; preclitellarly, aa a little longer than bc ($aa = 1\frac{1}{2} bc$); postclitellarly, aa subequal to bc .

Male pores (Fig. 1, a) small, slit-like, bounded by prominent anterior and posterior lips, placed on 10/11, about midway between setal lines of

b and *c*. Epidermis both anterior and posterior to each male pore is prominently elevated, glandulated and whitened; the intersegmental furrow runs into the thickenings until it reaches the slit showing the male pore. The general appearance of the male pore-region somewhat resembles that of *Dr. nemora*.

Spermathecal pores, 1 pair in 7/8, just medial to *c*-line. The pores were not definitely identified, but the region is slightly depressed to form a slit showing a glandular appearance. (Fig. 1, a).

Genital papillae absent; but, the epidermis anterior to the male pore-elevation is elevated slightly and irregularly.

Internal anatomy:

Septa 5/6-8/9 much thickened; the following ones either thin or slightly thickened. Gizzards 4 in XIII-XVI; the first one is poor in muscular development. Ovisacs small, club-shaped (extending only into XII); ovarian chamber dorsally closed off and ventrally slightly spaced; no ripe ova were found in either ovisacs or ovarian chamber. Testis sacs, 1 pair, small, oval in shape (without any constrictions), suspended by 9/10, the larger part lying in X. Duct rather long, coiled, most of it placed in IX, directly entering into the anterior face of prostata in X. Prostates small, pear-shaped, bluntly pointed ectally, with relatively thick glandular investment; central body ovoidal.

Spermathecae (Fig. 1, b), 1 pair, behind 7/8; ampulla small, elongate-ovoidal in shape; duct slender and long, coiled in its entire course, no atrial dilatation is found at its ectal end or in the parietes. Ampullae on both sides are bound to each other at their apices by a filament of the connective tissue.

Locality and material: Caroline Islands, Ponape, Kolonia, 1 semiclitellate specimen, July 25, 1939, collected by Prof. ESAKI.

Distribution: India, Burma, Lombok, Philippines, Caroline Islands (Yap and Ponape).

Remarks:

Unfortunately the characteristics of the dorsal pores were not identified definitely. Anteriorly to each male pore-elevation, an indistinctly demarcated epidermal elevation was found. Such elevations have been known in the case of *f. impertuosa* also. But, these are not identical with the genital papillae which are found in the present genus, being not connected with any kind of accessory glands internally.

2. *Drawida* sp.

Locality and material: Palau Islands, Korrör, several specimens, August 24, 1939, collected by Prof. ESAKI.

Remarks:

As the specimens had been extremely macerated, the writer was unable to identify the species. But, the present specimens seem to show a species distinct from *Dr. barwelli*.

3. *Pheretima taitensis* (GRUBE) 1866

(Fig. 2)

1866 *Perichaeta taitensis*, GRUBE, Verh. Ges. Wien, XVI, p. 180.

1900 *Pheretima taitensis*, MICHAELSEN, Tierreich, p. 308.

1913 *Pheretima taitensis*, MICHAELSEN, Nova Caledonia, Zool., 4, 5, pp. 267-271, fig. 6, A-E.

Description (based on the Ponape-specimen):

External characteristics: Colour, dorsally brownish violet with whitish setal zone, ventrally lighter than the dorsal surface or brownish pale, clitellum light chocolate. Body-length 166 mm, greatest diameter 5.5 mm, number of segments 107. Prostomium, epilobous ca. 1/4. First dorsal pore in 12/13. Clitellum entire, in XIV-XVI, without setae.

Setae moderate in size, slightly enlarged on III-IX and on the hinder part of the body; no marked difference is found in size and also none in interval between dorsal and ventral setae. Both mid-dorsal and -ventral breaks slight if present. Setal number as follows: 32/III, 49/IX, 61/XX, spermathecal setae 9/VII, 10/VIII, 11/IX, male pore setae 12.

Male pores (Fig. 2, a) situated ventrolaterally on XVIII; each pore transversely slit-like, on a small, circular papilla which is placed on an inconspicuous, whitish epidermal elevation. The elevation occupies about the posterior 2/3 of the segment and extends over a very little into XIX beyond 18/19. The pores are about 3/11 of the circumference apart from each other.

Spermathecal pores (Fig. 2, b), 21 pairs in 7/8 and 8/9; each pore minute, on a small papilla-like posterior lip of the preceding segment. (When the spermatheca is pulled off from the parietes internally, the lip-like porophore is found attached to the distal end of the ampullar duct.) The epidermis around the spermathecal porophore is slightly glandulated and appears to be whitish. The pores are about 1/5 of the circumference apart from each other.

Genital papillae are absent in the spermathecal region. The arrangement of the papillae in the male pore-region almost agrees with the MICHAELSEN's "*upoluensis*-Anordnung vorherrschend" (1913, p. 268, fig. 6). The number and position of the papillae are as follows (Fig. 2, a): (1) two, midventral, unpaired, presetal ones on both of XVII and XVIII;

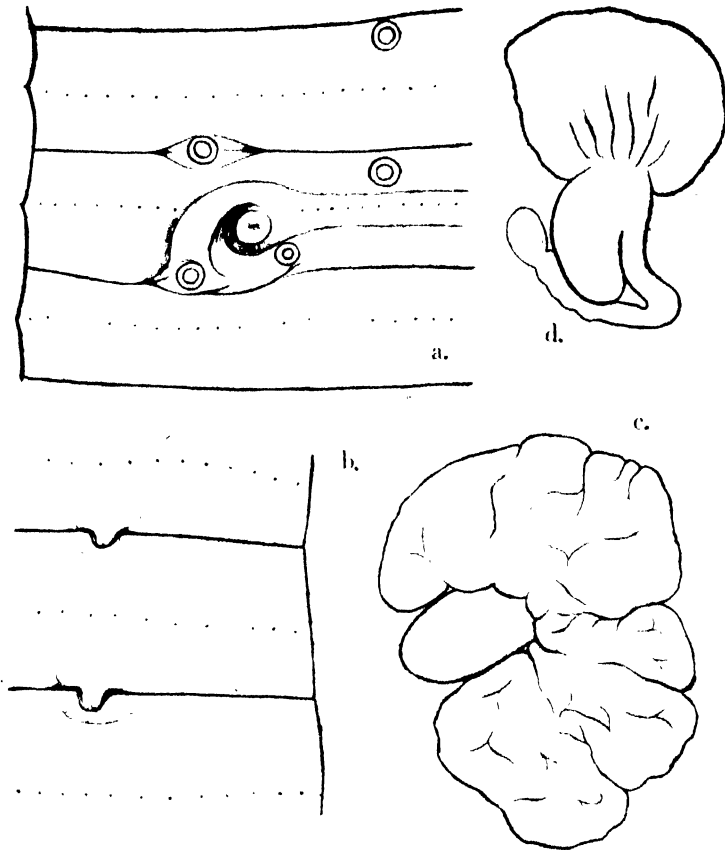


Fig. 2. *Pheretima taitensis*. a, ventral view of XVII-XIX; b, ventrolateral view of VII-IX, showing spermathecal pores; c, prostate gland with duct; d, a spermatheca. a-d, \times ca. 12.

(2) two pairs on both intersegmental furrows of 17/18 and 18/19, immediately lateral to male porophore-line; (3) one pair on XVIII, postsetal, just medial to male porophore. These papillae are all similar in shape, circular and centrally depressed; those of (3) are very little smaller than the others. Those of (3) and of the posterior pair in (2) are situated

on the margin of the elevation bearing the male porophore; the epidermis around the papillae is slightly elevated.

Internal anatomy:

No septa is especially thickened; 5/6, 6/7, 10/11-12/13 moderately thickened, 8/9 ventrally traceable, 9/10 absent. Intestine begins to swell in XVI. Intestinal caeca in XXVII, simple, finger-shaped, each extending anteriorly into about XXIII or XXIV, smooth on both dorsal and ventral margins but with septal constrictions. Hearts in XI-XIII, large in calibre; those in X vestigial. Lymph glands not found.

Testis sacs ventrally 2 pairs in X and XI, large and voluminous; those in each segment are separated from each other though rather closely placed; those of the same side are separated also. Seminal vesicles, 2 pairs in XI and XII, moderately large; dorsal lobe of each vesicle relatively small. Pseudovesicles large, 2 pairs, behind 12/13 and 13/14, a little larger than the dorsal lobe of the seminal vesicle.

Prostate glands small, in XVII- $\frac{1}{2}$ XIX, each consisting of a few thin lobes; duct very short but relatively thick with muscular shimmer, and may be said to be elongate-ovoid in shape. (Fig. 2, c). Copulatory chamber absent. Accessory glands small, circular in shape, each with a short stalk.

Spermathecae (Fig. 2, d) moderate in size, 2 pairs in VIII and IX, the diverticula of the anterior pair placed in front of 7/8. Ampulla saccular in shape, somewhat flattened dorsoventrally; duct thickly muscular, nearly equal to or a little shorter than the ampulla, sharply marked off from the latter. Diverticulum arising from about the ental third of the duct, curved in a V-shape; the ectal limb which corresponds to the stalk and which is a little shorter than the ental, is directed downwards (towards the parietes), and the ental limb which corresponds to the seminal chamber is directed upwards. The seminal chamber is a little thicker than the stalk though the lumen is large the wall being thin, and is weakly constricted in a moniliform, the entalmost sac is much larger than the rest. Diverticulum is nearly equal in length to the combined length of the ampulla and duct.

Localities and materials: Caroline Islands, Ponape, Kolonia, 1 clitellate specimen, July 25, 1939, collected by Prof. ESAKI. Caroline Islands, Truk, Tol Olej-Foup, 3 clitellate and 1 aclitellate specimens, April 11, 1940; Truk, Pata Sabote, 1 aclitellate specimen, April 4, 1940; Marianna Islands, Saipan, Tapochó, 1 clitellate specimen, May 6, 1940; Pagan, Songsong, 1 aclitellate specimen, April 25, 1940, collected by Messrs.

YASUMATSU and YOSHIMURA.

Distribution: Hermit Island, New Hebrides Tonga Island, Marshall Islands — Jaluit, Society Islands — Tahiti, Loyalty Island, Banks Islands, Samoa Islands, St. Mathias Island, Squally Island, Fiji Islands, Paumotu Islands, Steward Island, Cook Islands, Caroline Islands — Ponape and Truk, Marianna Islands — Saipan and Papan.

Remarks:

The arrangement of the genital papillae seen in the specimens of both Caroline and Marianna Islands is almost identical with that mentioned by MICHAELSEN in the terms of "*upoluensis*-Anordnung vorherrschend". Testis sacs in the anterior segment are separated from each other though they are closely situated at their posteromedial sides. Those in the posterior segment are rather widely separated from each other. Those of the same side are distinctly separated from each other by the septum 10/11 moderately thickened. Testes are large, and are seen from the dorsal side to be placed anterodorsally within the sac. The structure of the testis sacs, which has been repeatedly discussed by BEDDARD, UDE and MICHAELSEN, was rather clearly observed in the present specimens. This may possibly be due to the fact that the specimens were slightly macerated.

4. *Pheretima padasensis* (BEDDARD et FEDARB) 1895

(Fig. 3)

1895 *Perchaeta padasensis*, BEDDARD & FEDARB, Ann. Nat. Hist., VI, 16, p. 73.

1900 *Pheretima padasensis typica*, MICHAELSEN, Tierreich, p. 290.

1900 *Pheretima padasensis*, BEDDARD, P. Z. S., London, p. 628.

Description:

External characteristics: Colour, dorsally virescent brown with whitish setal zone, concentrated anteriorly, ventrally lighter than the dorsal (the colouration becomes gradually lighter towards the ventral surface); greenish blue iridescence is seen on both dorsal and ventral surfaces; clitellum chocolate. Body-length 81-164 mm, greatest diameter 4-6 mm, number of segments 110-116. Prostomium, epilobous ca. 1/3-2/3. First dorsal pore in 12/13; in most of the specimens this pore is indistinct and appears to be non-functional.

Setae moderately large, those found in the hinder part of body slightly enlarged; ventral ones more closely spaced than the dorsal. Midventral breaks usually lacking, and middorsal breaks slight or only partly distinct. Setal number as follows: 28-32/III, 35-43/IX, 36-44/XX, spermathecal

setae 12-14/V, 13-15/VI, 13-16VII, 14-16/VIII, 14-16/IX, male pore setae 8-10.

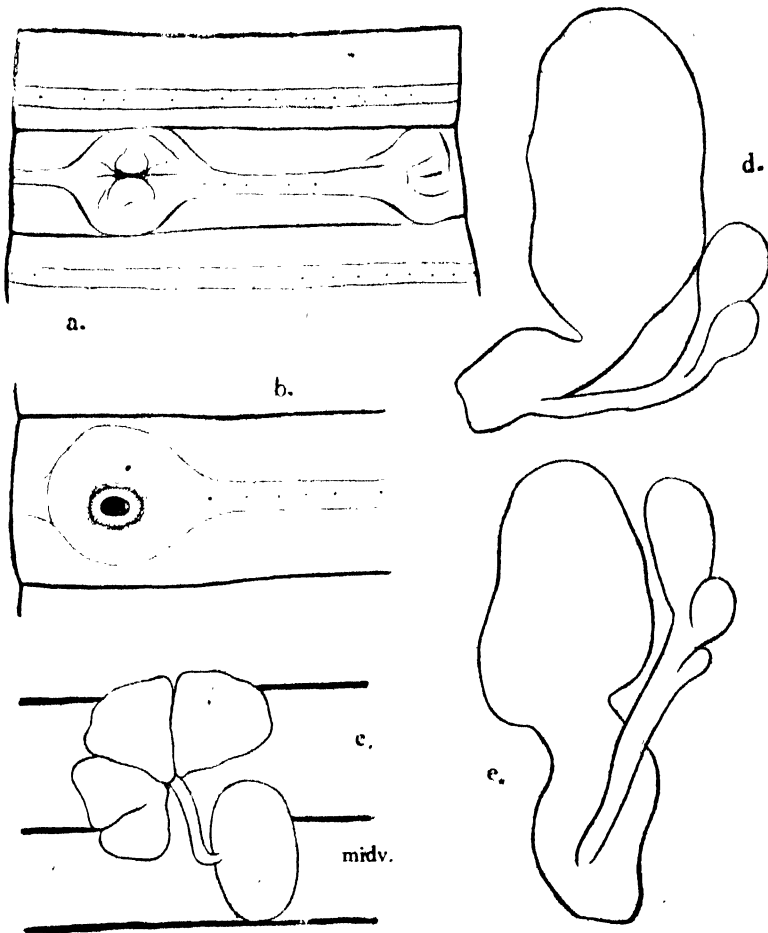


Fig. 3. *Pheretima padasensis*. a & b, male pores, a-closed, b-opened, \times ca. 14.4; c, a prostate gland with duct and copulatory chamber, drawn with free hand; d & e, spermathecae, \times ca. 33.6.

Clitellum entire, in XIV-XVI, without setae and dorsal pores. The glandularity of both anterior and posterior ends of the clitellum is not completely developed in all the clitellate specimens which appear to be fully matured.

Secondary male pores (Fig. 3, a & b) small, transversely eye-shaped or slit-like, each surrounded by slightly elevated whitish ridge (when

closed); each pore is situated on an inconspicuous epidermal elevation which occupies about one segment anteroposteriorly. The pores are about $1/4$ of the circumference apart from each other.

Spermathecal pores, 4 pairs in 5/6–8/9; each pore minute and epidermal; the portion around the pore is weakly glandulated and whitened. The pores are about $2/7$ of the circumference apart from each other.

Genital papillae are absent.

Internal anatomy:

No septa is thickened especially; 5/6 and 6/7 moderately thickened, 8/9 ventrally traceable, 9/10 absent. Intestine begins to swell in XV; caeca in XXVII, simple, finger-shaped, each extending anteriorly into about XXIV or XXIII, smooth on both ventral and dorsal margins. Hearts absent in X (but, vestigial ones were found in one specimen); those in XI–XIII large in calibre. Lymph glands not found.

Testis sacs ventrally 2 pairs in X and XI, moderate in size; those in each segment separated from each other though rather closely placed; those of the same side separated also. Seminal vesicles, 2 pairs in XI and XII, either small or very small, vesicular on surface; each with a relatively large, distinctly constricted, triangular or conical, smooth-surfaced dorsal lobe. Pseudovesicles, 1 pair, very large, nearly equal to or a little smaller than the seminal vesicle, similar in shape to the latter provided with a distinctly constricted dorsal lobe, but uniformly smooth on surface.

Prostate glands small and thin, each consisting of a few lobes, somewhat fan-shaped, in XVII–XVIII or a part of XVIII; duct short and thin but with muscular shimmer, nearly straight or weakly curved in S-shape with equal thickness. The duct enters into the lateral side of an anteroposteriorly oval, cushion-like protruded copulatory chamber which occupies a little more than one segment (Fig. 3, c).

Spermathecae (Fig. 3, d & e) very small; ampulla ovoidal in shape; duct relatively thick, entally narrowing, a little shorter than the ampulla. Diverticulum arising from the ectal third of the ampullar duct, a little shorter than the main portion; the stalk is slender but thick-walled, subequal in length to the ampullar duct, and the seminal chamber is thin-walled and is dilated at the ental end forming a small oval sac. In most of the cases examined, the diverticulum is provided with 1–3 accessory diverticula. They are branched at the ental portion of the stalk and form there variously-sized swellings which are either knob-like or spatula-like.

Locality and material: Caroline Islands, Ponape, Kolonia, 5 clitellate

and 2 semi-clitellate specimens, July 25, 1939, collected by Prof. ESAKI.

Distribution: Borneo, Celebes, Caroline Islands.

Remarks:

The terms of "*Seminal vesicles* in XIII" (BEDDARD & FEDARB '95, MICHAELSEN '99 & '00) must be changed into "*pseudovesicles*", because these organs are not connected with any of the testis sacs. These large pseudovesicles were constantly found in the present specimens. The characteristic status of the dorsal lobe of the seminal vesicle is also constant in these specimens. By these distinct characteristics together with other minor ones *Ph. padasensis lokonensis* may possibly be specifically distinct from f. *typica* as BEDDARD ('00) revised.

5. *Pheretima sangirensis* (MICHAELSEN) 1891

(Fig. 4)

1891 *Perichaeta sangirensis*, MICHAELSEN, Mitt. Mus. Hamburg, VIII, p. 36.

1900 *Pheretima sangirensis typica* & *crassicystis*, MICHAELSEN, Tierreich, p. 301.

1924 *Pheretima sangirensis*, MICHAELSEN, Treubia, V, p. 113.

Description:

External characteristics: Colour, dorsally dark virescent brown with whitish setal lines, concentrated anteriorly; ventrally light brown to pale; clitellum deep chocolate. Of the most well-preserved specimens, body-length 135 mm, greatest diameter 5.5 mm; number of segments 98-104. Prostomium, epilobous ca. $1/3-1/2$. First dorsal pore in 11/12 distinct and functional. Clitellum entire, in XIV-XVI, without setae.

Setae small, those on III-VIII and on the hinder part of the body slightly enlarged; no marked difference is found in size or in interval between dorsal and ventral setae; both mid-dorsal and -ventral breaks present but are slight. Setal number as follows: 28/III, 40-41/IX, 62-67/XX, spermathecal setae 14-16/VIII, 15-16/VIII, male pore setae 8-12.

In all specimens the penis is protruded a little exposing the head-region from the small, somewhat oval-shaped secondary male pore provided with moderately elevated and slightly wrinkled ridge. The elevation occupies a length a little shorter than that of one segment measured anteroposteriorly. (Fig. 4, a). The pores are about $1/4$ of the circumference apart from each other.

Spermathecal pores, 1 pair in 7/8; each pore minute, on a tiny tubercle which is placed in an inconspicuous, oval-shaped depression found in the intersegmental furrow; the margin of the depression is slightly glandulated

and whitish in colour. The pores are about $1/3$ of the circumference apart from each other.

Genital papillae are absent.

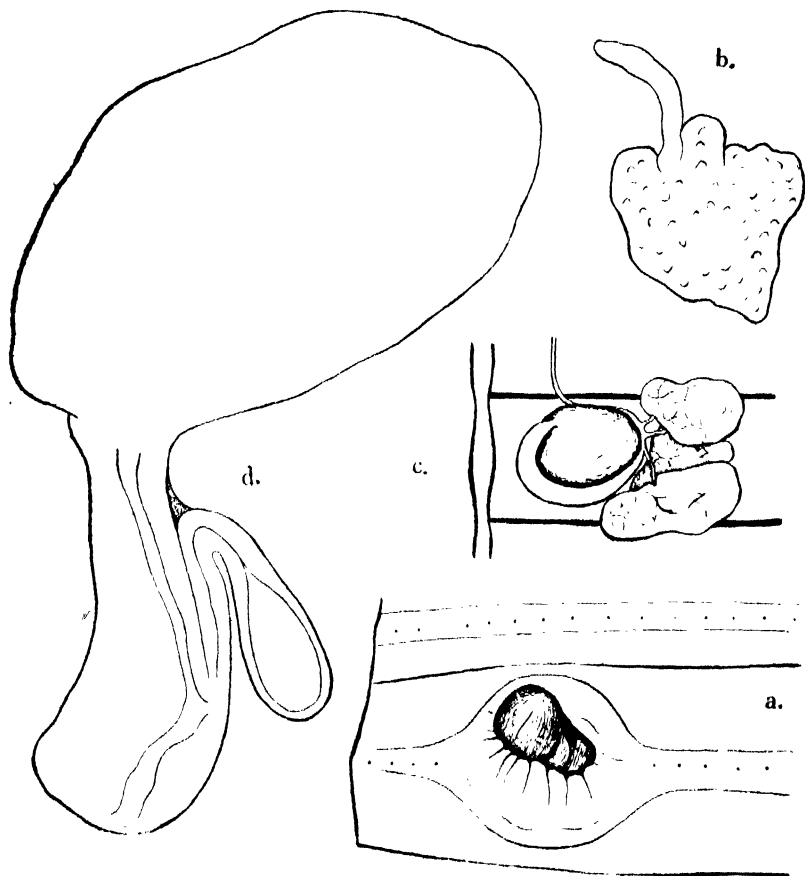


Fig. 4. *Pheretima sangirensis*. a, ventrolateral view of male pore-region, \times ca. 14.4; b, a seminal vesicle with finger-shaped dorsal lobe, \times ca. 14.4; c, prostate gland with duct and copulatory chamber, drawn with free hand; d, a spermatheca with diverticulum, of which the most part of the stalk is developed within the ampullar duct, \times ca. 33.6.

Internal anatomy:

No septa is thickened especially; 5/6 and 6/7 moderately thickened, 8/9 ventrally traceable, 9/10 absent. Intestine begins to swell in XV. Intestinal caeca in XXVII, simple, finger-shaped, each extending anteriorly into about XXIV, ventrally provided with several serriformed outgrowths

in its basal portion. Hearts in X-XIII; those in X smaller than the rest. Lymph glands small, found behind caecal segment caudalwards.

Testis sacs ventrally 2 pairs in X and XI, large, almost reaching the lateral sides of the gut; those in each segment separated and those in the same side separated also from each other. Seminal vesicles, 2 pairs in XI and XII, small and thin, black in colour, vesicular on surface; dorsal lobe of each vesicle slender and long or finger-shaped - its status is characteristic and is constant at least in the four specimens opened (Fig. 4, b). Pseudovesicles, 1 pair, behind 12/13, vestigial in size.

Prostate glands small and thin, each consisting of 3 lobes which are connected by fine ducts (these lobes may sometimes be distinctly separated from one another), occupying only a little more than one segment. Duct short, moderate in thickness, with nearly equal thickness throughout or ectalwards becoming gradually thicker in a very slight degree, curved in a bow-shape along the posterior margin of the copulatory chamber (Fig. 4, c). The duct with much decreased thickness, enters into the medial side of the copulatory chamber which is provided with thick muscular wall, and pierces through the club-shaped penis which is weakly bent dorsalwards and which fills almost all of the narrow lumen of the chamber. The copulatory chamber is single, transversely oval in shape, protruded in cushion-like manner into the coelom, occupying about 2/3 of one segment measured anteroposteriorly.

Spermathecae (Fig. 4, d) small, 1 pair in VII, just in front of 7/8. Ampulla ovoidal or pear-shaped; duct relatively thick, a little shorter than the ampulla, not so distinctly marked off from the latter. Diverticulum arising from the distal third of the ampullar duct, is about $\frac{1}{2}$ of the combined length of the ampulla and duct. The stalked portion of the diverticulum is shorter than the ampullar duct, and its greater part is fused with or developed within the latter; in the latter case the ental portion of the stalk may be connected with the duct by a filament of fine connective tissue. The seminal chamber is small but is clearly dilated from the stalk, ovoidal in shape, shorter than the latter and is free from the ampullar duct.

Localities and materials: Palau Islands: Korrör, 7 clitellate specimens (the other several macerated specimens may possibly belong to the present species), August 24, 1939; Babeldaob, Ngiwal — Ngarard, 2 clitellate and 2 a clitellate specimens, August 16, 1939; Babeldaob, Ngarard, 5 clitellate and 5 a clitellate specimens, August 17, 1939, collected by Prof. ESAKI.

Distribution: Lobo, Moluccas, Palau Islands.

Remarks :

BEDDARD ('00) united *Ph. sangirensis* (MICHAELSEN '99) with *Ph. montana*. But, the writer thinks that although the shape of the copulatory chamber may be variable to some extent it is more advisable to separate specifically the forms which have a single chamber from those with compound ones. BEDDARD himself remarked in his paper that the forms with a single chamber should be classified as the second group against the first group (= *Ph. montana*-group).

6. *Dichogaster bolau* (MICHAELSEN) 1891

- 1891 *Benhamia bolavi* (corr. *bolau*), MICHAELSEN, Mitt. Mus. Hamburg, VIII, p. 9, Pl. figs. 1 & 2.
 1893 *Benhamia bolau*, HORST, Weber, Reise Niederl. O.-Ind., III, p. 37, Pl. 2, fig. 14.
 1895 *Benhamia octonephra*, ROSA, Boll. Mus. Torino, X, 204, p. 2.
 1895 *Benhamia bolau*, ROSA, Mem. Acc. Torino, II, 45, p. 137, Pl. fig. 13.
 1896 *Benhamia bolau palmicola*, EISEN, Mem. Calif. Ac., II, 5, p. 132, Pl. 46 & 48-50.
 1897 *Benhamia bolau*, MICHAELSEN, Mitt. Mus. Hamburg, XIV, p. 15.
 1900 *Benhamia pacifica*, EISEN, P. Calif. Ac. III, 2, p. 209, Pl. 10, fig. 68-93.
 1900 *Dichogaster bolau*, MICHAELSEN, Tierreich, p. 340.
 1938 *Dichogaster bolau*, CHEN, Contr. Biol. Lab. Sci. Soc. China, XII, Zool., 10, p. 419.
 1940 *Dichogaster bolau*, KOBAYASHI, Sci. Rep. Tôhoku Imp. Univ., Biol., XV, 1, p. 1
 (see this paper for complete bibliography in 1900-1938).

Localities and materials : Caroline Islands, Ponape, Kolonia, 4 clitellate and 1 a clitellate specimens, July 13 & 25, 1939, collected by Prof. ESAKI. Truk, Tol Olej-Foup, 1 clitellate and 1 a clitellate specimen, April 6, 1940; Truk, Pata Sabote, 1 a clitellate specimen, April 4, 1940, collected by Messrs. YASUMATSU and YOSHIMURA. Palau Islands, Babeldaob, Eimilik-Ngarumisukan, 1 clitellate specimen, August 18, 1939, collected by Prof. ESAKI.

Distribution : Africa, Madagascar, India, Burma, California, Mexico, Central and S. America, W. Indies, New-Caledonia, Loyalty Islands, Marianna Islands, Caroline Islands, Palau Islands, Philippine Islands, Malay States, Hainan, (Germany — Hamburg).

7. *Pontodrilus* sp.

Locality and material : Marianna Islands, Saipan, Garapan-Sagod Tasi, 3 clitellate specimens, May 5, 1940, collected by Messrs. YASUMATSU and YOSHIMURA. The specimens were collected from a mangrove-garden that was formerly sea-shore.

External characteristics: Colour in alcohol, dorsally brown with reddish and purplish tinges, ventrally a little lighter than the dorsal surface. Body-length 50-55 mm, greatest diameter 1.8-2.0 mm, number of segments ca. 100. As the head end is extremely macerated, it is difficult to determine the type of the prostomium; possibly it may be zygorobous. Setae small, widely paired; setal distance approximately measured at the portion slightly posterior to the male pore-region as follows: $aa=bc=1\frac{1}{2}$ $cd=2$ $ab=2/3$ dd ; dd subequal to $1/4$ u . Setae a and b of XVIII are displaced close to the male pore, and are degenerated in size being as short as about $\frac{1}{2}$ of the usual setae; these setae are however not especially modified, and may be hardly visible externally. Clitellum is not yet developed. Male pores, 1 pair on XVIII, between a and b ; each pore slit-like, in a low V-shape, placed on a slight, whitish and glandulated epidermal elevation occupying about one segment antero-posteriorly. Female pores, 1 pair, just ventral to a ; these are hardly recognizable. Spermathecal pores, 2 pairs in 7/8 and 8/9, in line with b ; each pore minute, placed on a very small tubercle which is surrounded by a small, circular and slightly glandulated rim. No genital papillae are found elsewhere.

Internal anatomy:

Septum 4/5 thin, 5/6-8/9 slightly thickened, 9/10-11/12 moderately thickened. In one specimen, the septa are slightly thicker than those above mentioned. Oesophagus is dilated in V though its wall is not thickened. In the specimen with thicker septa, the oesophagus in V and VI is dilated, and its wall in V is very slightly thickened. Last heart in XII. Nephridia meganephridic, beginning in XIII. Testes free in X and XI; funnels are found also in X and XI. The testes are found ventrally behind 9/10 and 10/11 as very small whitish bodies; sperm-ducts (parts of vasa efferentia) were easily identified. Seminal vesicles, 2 pairs in XI and XII, behind 10/11 and 11/12, small, bunchy; the posterior pair may be a little smaller than the anterior. The glandular portion of the prostate has been macerated, but the duct is well-preserved. The duct is well-musculated through its entire course, relatively long, curved in L-shape with slightly thinner ends; close to its ectal end are found the setae a and b (as already described). Although it is difficult to determine the original status of the glandular portion, its approximate feature may be judged as follows. It is tubular, slightly convoluted and thinner than the duct, but it may be a little longer than the latter if stretched. The poor development of the glandular portion

may be due mainly to the immaturity of the specimen. Possibly the sperm-duct enters into the ental end of the gland on each side. Spermathecae, especially those in the anterior pair, are not yet well-developed. They are very small; ampulla club-shaped with a little thicker ental end; duct thick, muscular and not sharply marked off from the ampulla, its ectal part becoming thinner. Diverticulum arising from the portion slightly higher than the ectal end of the duct, slenderly tubular, a little shorter than the main portion, the ental part being very slightly dilated. (In one specimen the diverticula of the anterior pair were longer than the main portion though the ampullae were yet very poorly developed.)

Remarks :

As all the specimens have been macerated and are yet immature, the writer was obliged to postpone the establishment of the new form, though they seem to be a distinct species, or at least may be a distinct subspecies of *P. agnesae* STEPHENSON which is a unique terrestrial form among the genus. From *P. agnesae* the present species differs mainly in the presence of the setae *a* and *b* of XVIII (though they are degenerated) and in the position and shape of the seminal vesicles.

CONCEPTACLE DEVELOPMENT OF TWO SPECIES OF SARGASSUM OF THE SUBGENUS MICRANCANTHA¹⁾

By

MASATO TAHARA

Biological Institute, Tōhoku Imperial University, Sendai

(With 2 text-figures)

(Received May 30, 1941)

The genus *Sargassum* is divided into six subgenera, namely *Phyllotricha*, *Schizophycus*, *Eusargassum*, *Bactrophycus*, *Micracantha* and *Arthrophycus*. Last year the present writer investigated the conceptacle development of thirteen species of *Sargassum*. Among them nine belong to *Bactrophycus*, one to *Phyllotricha*, one to *Schizophycus* and two to *Eusargassum*. In the spring of this year, the writer had an opportunity to collect the material of two species of the subgenus *Micracantha*. The conceptacle development of these species will be described in the present paper. Thus now only the species of the subgenus *Arthrophycus* remain uninvestigated; these species grow in warmer regions and it is not easy for the present writer to obtain the material for the investigation of these plants.

As was stated in the writer's two previous papers²⁾, the species of the subgenus *Bactrophycus* show a marked peculiarity in the early stage of the conceptacle development, that is, in these plants the tongue cell becomes detached from the wall of the conceptacle and is transferred to the ostiole, thus for a time completely or partially closing up the opening of the conceptacle. The species of the subgenera, *Phyllotricha*, *Schizophycus* and *Eusargassum* do not share this peculiarity.

The subgenus *Micracantha* has in appearance a close resemblance to the subgenus *Bactrophycus*. So it will be interesting to know whether

¹⁾ The cost of this research has been defrayed from the Scientific Research Expenditure of the Department of Education.

²⁾ TAHARA, M. 1940:— On the Development of the Conceptacle of *Sargassum*, *Cocophora* and *Cystophyllum*. Sci. Rep. Tōhoku Imp. Univ. Vol. XV. No. 3. TAHARA, M. 1941:— Further Studies on the Conceptacle Development of *Sargassum*. Sci. Rep. Tōhoku Imp. Univ. Vol. XVI. No. 1.

the translocation of the tongue cell occurs or not in the species of this subgenus. In the species of this subgenus the receptacles are complanated, ovate, excavated on one surface and longitudinally elevated on the other, and minutely dentated on the margin or at the apex. Along the coast of Japan we have only two species of this subgenus.

1. *Sargassum micracanthum* (Kütz) YENDO

The material of this alga was obtained from Kesenuma, Miyagi Prefecture, through the kindness of Mr. E. HIRAI. The fresh materials were sent from this town to Sendai and were fixed there by chrom-acetic solution. The plants collected on February 25th of this year were very suitable for the present investigation. Cross and longitudinal sections through the tip of young shoot, 3-5 μ in thickness, were stained HEIDENHAIN's haematoxylin and light green. Text-fig. 1 shows the main outline of the conceptacle development of this plant. As is usual in the species of *Sargassum*, the initial cell of the conceptacle is at first divided transversely into two cells by a curved wall, the upper cell forming the tongue cell. The lower cell repeats longitudinal divisions to form the basal portion of the conceptacle. The arrangement of the cells around the tongue cell is radial. In early stages of the development, for a tolerably long time, the tongue cell remains attached to the wall of the conceptacle (Fig. 1, c, d); but in a more advanced stage the dislocation of the tongue cell takes place also in this plant (Fig. 1, e). Thus the conceptacle development of this alga proceeds in a manner intermediate between the primitive type, to which *Phyllotricha*, *Schizophycus* and *Eusargassum* belong, and the *Bactrophycus*-type.

2. *Sargassum nigrifolium* YENDO

The material of this alga was collected by the writer on May 16th of this year at Enoshima, Kanagawa Prefecture. The conceptacle development of this plant is just the same as that of the preceding species. Fig. 2, a is an early stage of the conceptacle development; the tongue cell is seen in the middle of the conceptacle. Fig. 2, b is a later stage, where the tongue cell, still fixed to the wall of the conceptacle, already shows a sign of degeneration. But in a still later stage the tongue cell becomes detached from the wall of the conceptacle and is transferred to the ostiole (Fig. 2, c). Fig. 2, d is a more advanced stage; the tongue cell is seen in the middle of the ostiole.

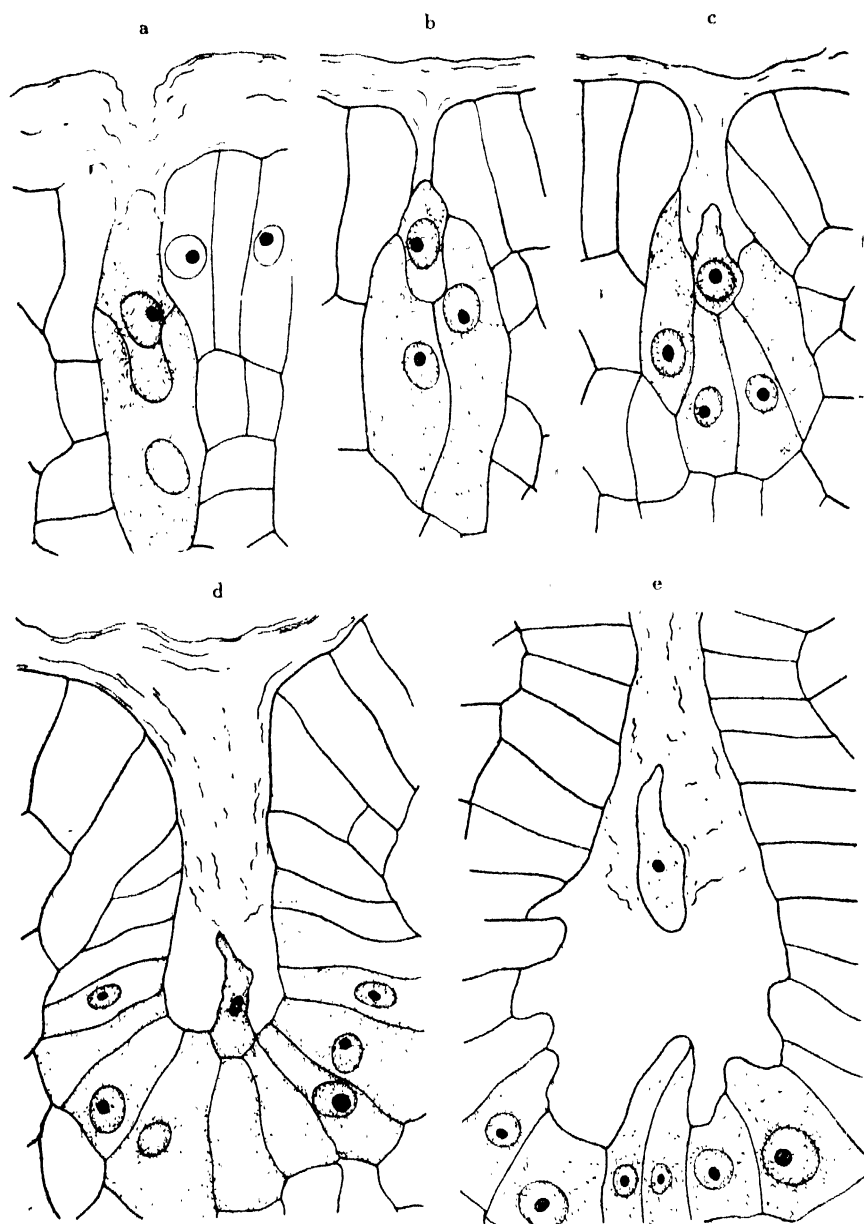


Fig. 1. Conceptacle development in *Sargassum micracanthum*. a, initial cell is divided into two cells by a curved wall, the upper cell being the tongue cell. b-d, basal cell is divided by longitudinal walls. The tongue cell remains attached to the wall of the conceptacle. e, a more advanced stage, where the tongue cell becomes detached from the wall of the conceptacle and is transferred to the ostiole. ($\times 900$)

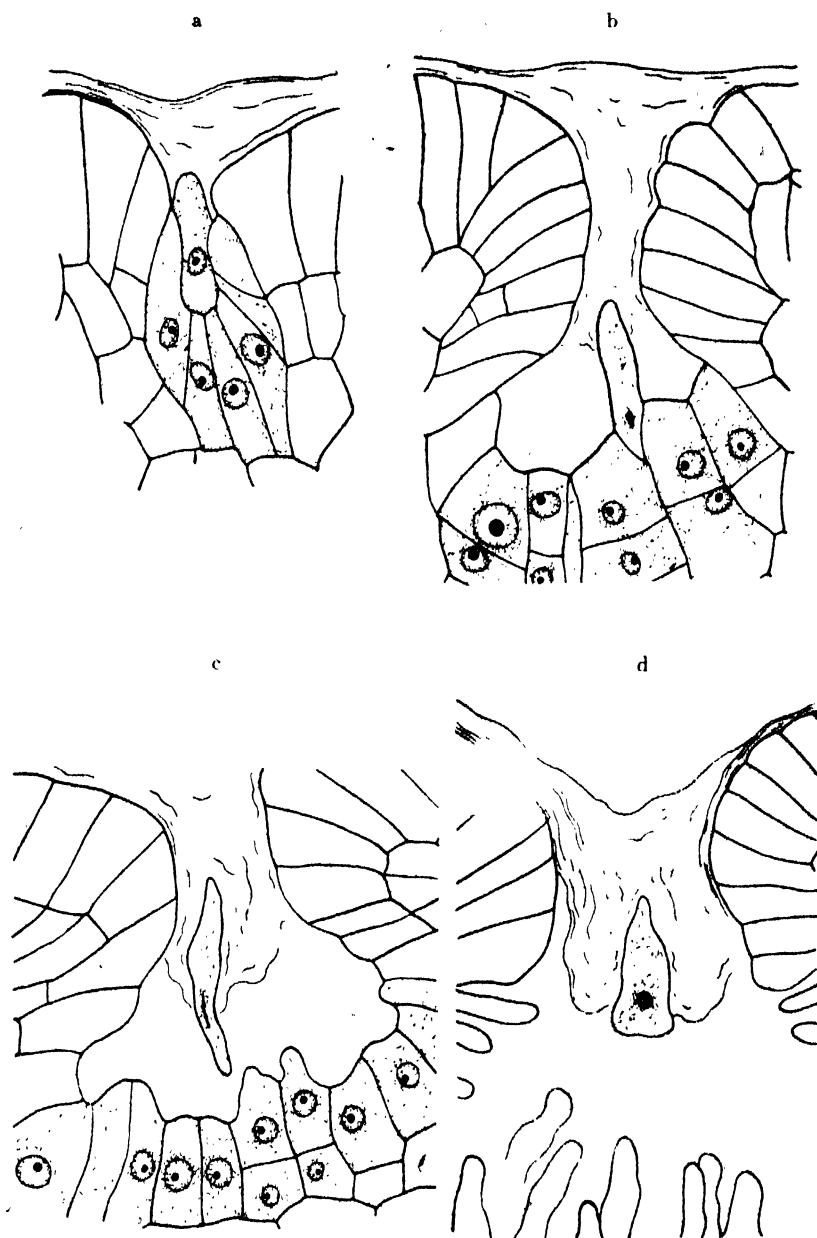


Fig. 2. Conceptacle development in *Sargassum nigrifolium*. a and b, early stages. The tongue cell is fixed to the wall of the conceptacle. c and d, later stages. The tongue cell is transferred towards the ostiole. ($\times 900$)

SUMMARY AND CONCLUSION

In the development of the conceptacle of two species of *Sargassum* of the subgenus *Micracantha*, namely *S. micracanthum* and *S. nigrifolium* the translocation of the tongue cell takes place in a rather later stage. The translocation of the tongue cell in an early stage of development is found, so far as the investigation has reached, only in the species of the subgenus *Bactrophycus*. The five subgenera of *Sargassum* may be arranged thus in the following order of complexity: *Phyllotricha*, *Schizophycus*, *Eusargassum*, *Micracantha*, and *Bactrophycus*.

STUDIES ON FRESHWATER BRYOZOA OF JAPAN II

FRESHWATER BRYOZOA OF TYÔSEN (KOREA)

By

MAKOTO TORIUMI

Biological Institute, Tôhoku Imperial University, Sendai, Japan

(With 13 text-figures)

(Received May 31, 1941)

INTRODUCTION

In spite of the fact that the freshwater Bryozoa are rather common in Tyôsen, this group of animals has been insufficiently studied up to the present time.

In 1938, Mr. NOBUO SASAKI of the Tôhoku Imperial University made a trip to Tyôsen for the purpose of collecting the freshwater sponges, and at that time he also obtained many specimens of freshwater Bryozoa which it was kindly suggested to the present writer should study. The present report deals with the material above alluded to and the forms determined are shown in the following list.

1. *Paludicella articulata* (EHRENBERG)
2. *Fredericella sultana* (BLUMENBACH)
3. *Plumatella repens* var. *emarginata* (ALLMAN)
4. *P. repens* var. *minuta* TORIUMI
5. *P. repens* var. *flabellum* (VAN BENEDEN)
6. *Hyalinella punctata* (HANCOCK)
7. *H. toanensis* HÔZAWA & TORIUMI
8. *Stephanella hina* OKA
9. *Pectinatella gelatinosa* OKA
10. *Lophopodella carteri* (HYATT)

All of the species mentioned above are these reported from Tyôsen for the first time.

I wish to express here my sincere thanks to Professor SANJI HÔZAWA for his kind supervision during the course of the present study. I am also obliged to Mr. NOBUO SASAKI for his kindness and generosity in giving me many valuable specimens which he had collected.

DESCRIPTION

1) *Paludicella articulata* (EHRENBERG)

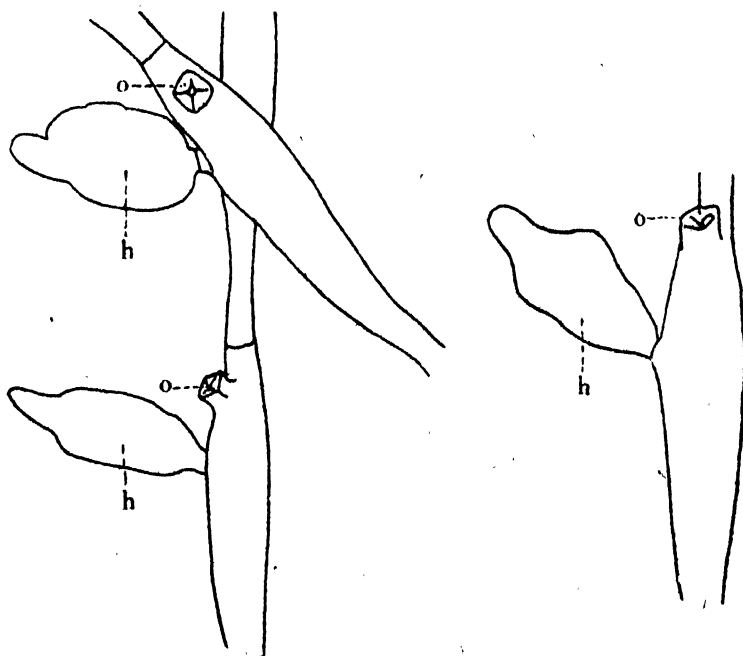
Alcyonella articulata, EHRENBERG, 1831.

Paludicella ehrenbergi, ALLMAN, 1856, pp. 113-115, Pl. X, figs. 1-5; VANGEL, 1894, p. 153; DAVENPORT, 1904, p. 215, Pl. VI, fig. 3; HARTMEYER, 1909, pp. 57-58, Figs. 128-129; KAWAMURA, 1918, p. 175, Fig. 499.

Paludicella articulata, ALLMAN, 1844, p. 331; KRAEPELIN, 1887, pp. 98-99, Pl. IV, fig. 107; HARMER, 1915, pp. 441-447, Pl. LXII, figs. 1-10; ROUSSELET, 1916, p. 141; ROGICK, 1935, pp. 248-249; 1940, p. 194, Pl. I, fig. 4, Pl. II, fig. 5; TORIUMI, 1941, p. 195.

The branches forming zoarium are rather sparse, being either recumbent or erect, and are given off almost at right angles from the wide part of the zooecium. The ectocyst is not encrusted, and is of a pale brown hue and transparent. The zooecia are claviform, elongate and very slender.

The tubular orifice (text-fig. 1, o) is squared at the tip and is placed distally with the respect to the wide part of the zooecium. The septa are present. It was impossible to examine the number of tentacles.



Text-fig. 1. Hybernacula of *Paludicella articulata*. h—hybernacula; o— orifice of the zooecium. ($\times 40$)

A few hibernacula (text-fig. 1, h) which are the shape of a short spindle are present at the wide part of the zooecium. The chitinous wall of the hibernacula is thicker than that of the zooecia, being yellowish brown in color, and transparent and elastic.

This species was found in Bukeiko, Kankyôhokudô and Tyôzya-ga-ike, Zenranandô.

2) *Fredericella sultana* (BLUMENBACH)

Tubularia sultana, BLUMENBACH, 1779.

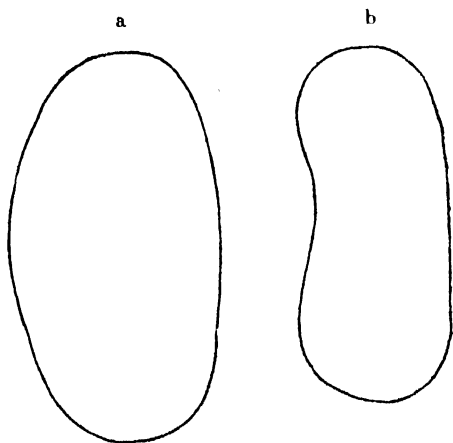
Fredericella sultana, ALLMAN, 1844, p. 331; 1856, pp. 110-111, Pl. IX, figs. 1-7; HANCOCK, 1850, p. 173; KRAEPELIN, 1887, pp. 103-104, Pl. VII, fig. 138; VANGEL, 1894, p. 153; DAVENPORT, 1904, p. 216; ROUSSELET, 1907, p. 251; 1916, p. 141; HARTMEYER, 1909, p. 55, Figs. 121, 122; ANNANDALE, 1910, p. 39; HARMER, 1915, pp. 448-449, Pl. LXIII, figs. 11-14; KAWAMURA, 1918, p. 176; ROGICK, 1935, p. 250, Pl. XI., fig. 2; 1937, pp. 101-102, Fig. 1; 1940, p. 195, Pl. III, fig. 13; TORIUMI, 1941, p. 196.

The zoarium is recumbent, branching antler-like. The branching is very open.

The ectocyst is sandy in color, being encrusted. The zooecia are long, slender, nearly cylindrical and are strongly keeled. The septum, which is pale brown is rarely present at the base of the branch.

The polypides are degenerated.

The fixed statoblasts (text-fig. 2) are yellowish brown and the shape and the size of these are variable. There may also be seen bean-shaped, oval and almost quadrangular statoblasts. The length of the fixed statoblasts varies from 0.45 to 0.5 mm and the breadth is from 0.19 to 0.26 mm.



Text-fig. 2. Two fixed statoblasts of *Fredericella sultana*. ($\times 100$)

The specimens of this species was secured from Zyôsyaku-tyosuiti, Keisyonandô.

3) *Plumatella repens* var. *emarginata* (ALLMAN)

Plumatella emarginata, ALLMAN, 1844, p. 330; 1856, p. 104, PL VII, figs. 5-10; ANNANDALE, 1907, p. 177; 1910, p. 47; 1912, p. 140; HARTMEYER, 1909, p. 53, Fig. 117; HARMER, 1913, p. 453; VORSTMAN, 1928, pp. 4-5, Figs. 1, 2, Pl. I, figs. 1-4; HASTINGS, 1929, p. 130; 1929, p. 307.

Plumatella princeps var. *emarginata*, KRAEPELIN, 1887, p. 120, Pl. IV, fig. 108, Pl. V, fig. 123; DAVENPORT, 1904, p. 217, Pl. VI, fig. 5.

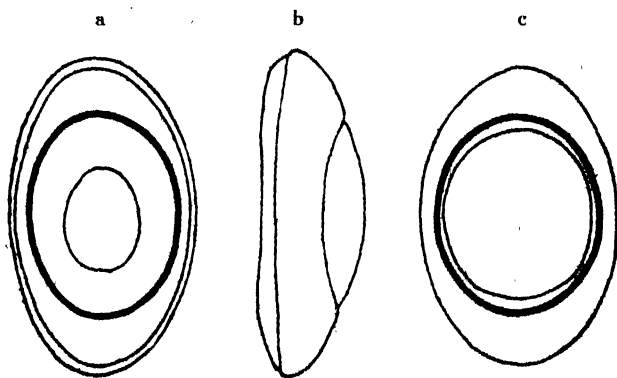
Plumatella emarginata forma *typica*, LEE, 1936, pp. 401-403, Fig. II.

Plumatella repens var. *emarginata*, VANGEL, 1894, p. 154; ROGICK, 1935, pp. 255-256, Pl. XLI, fig. 6; 1937, pp. 100-101; 1940, p. 198, Pl. I, figs. 1-3, Pl. III, figs. 11, 12; TORIUMI, 1941, p. 198, Fig. 3.

The zoarium is recumbent, and the branches are antler-like in form.

Sometimes the zoarium sends off several short branches. The ectocyst is encrusted and ranges in color from dark brown to sandy. The zooecia are long, nearly cylindrical, and are keeled. In the vertical branches the keel as a rule disappears. The septa are crescent-shaped and are of a deep brown or black color. The number of tentacles varies from 30 to 45.

The free statoblasts (text-fig. 3) are elongate and are sometimes almost oval, being rounded or subtruncated at both extremities. The annulus covers the greater part of the one face, and a small part of the other of the capsule. Usually the annulus is distinctly narrower on the sides than at the ends.



Text-fig. 3. Free statoblasts of *Plumatella repens* var. *emarginata*. a—dorsal view; b—side view; c—ventral view. ($\times 100$)

The free statoblasts are 0.31-0.43 mm in length, and 0.21-0.27 mm in breadth. The length of the capsule is between 0.24 and 0.3 mm and its breadth is between 0.18 and 0.23 mm.

The fixed statoblast is surrounded by chitinous lamella that is minutely serrated on the margin. The dorsal surface of the capsule is brown and is minutely mammillated. The capsule of the fixed statoblast is 0.32–0.41 mm in length and 0.23–0.29 mm in breadth. The breadth of the chitinous lamella varies from 0.02 to 0.03 mm.

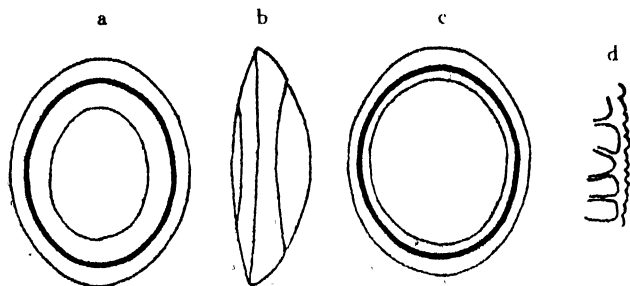
This variety was obtained from the following localities, viz.: Bukeiko, Kankyôhokudô; Kôho, Ryûko and Hanpoko, Kankyonandô; Masantei, Kôgendô; Hyôtan-ike, Keizyô-hu; Two small ponds at Seiryôri, Keizyô-hu.

4) *P. repens* var. *minuta* TORIUMI

Plumatella repens var. *minuta*, TORIUMI, 1941, p. 202.

The zoarium is recumbent, the branches being antler-like. This variety resembles *P. repens* var. *typica* very closely. The ectocyst is slightly swollen, soft, colorless, hyaline, and is more or less encrusted.

The keel is present obscurely. The septum is not observable in any part of the zoarium. The number of tentacles was not examined.



Text-fig. 4. Free statoblasts of *Plumatella repens* var. *minuta*.
a—dorsal view; b—side view; c—ventral view; d—serration on
margin of annulus. (a–c, $\times 100$ d, $\times 450$)

The free statoblasts (text-fig. 4, a, b, c) are oval nearly circular.

The annulus covers both faces of the capsule but in a slight degree.

The margin of the annulus bears a number of irregular minute processes (text-fig. 4, d) and they are visible when strongly magnified. The capsule is mammillated. The length of the free statoblasts is 0.27–0.28 mm and the width is 0.22–0.23 mm. The capsule is 0.22–0.23 mm long and 0.19–0.2 mm wide.

The fixed statoblast is absent.

This variety is not abundant in Tyôsen, and was secured from only one locality, Bukeiko, Kankyôhokudô.

5) *P. repens* var. *flabellum* (VAN BENEDEN)

Alcyonella flabellum, VAN BENEDEN, 1848; ALLMAN, 1850, p. 90.

Plumatella repens var. *flabellum*, ROGICK, 1934, p. 317; 1935, p. 245; 1937, p. 99.

Plumatella casmiana, OKA, 1907, Fig. 3, p. 117; VORSTMAN, 1928, Fig. 1, Fig. 5, Pl. I, fig. 8; TORIUMI, 1941, p. 203.

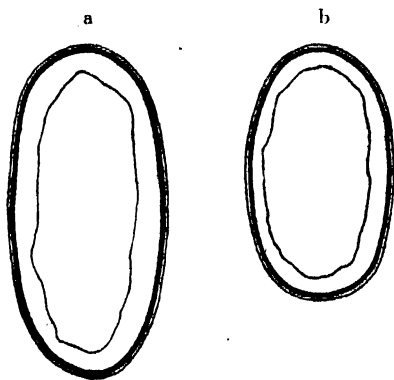
The zoarium is entirely recumbent, being branches densely. The branches are short and lie close to one another giving a compact appearance to the zoarium. The young zoarium appears flabelliform, but is seldom geminated.

The ectocyst is encrusted and ranges in color from yellowish brown to sandy, being either opaque or semiopaque. Sometimes near the distal end of the zooecium, the ectocyst is pigmented with a dark grey color.

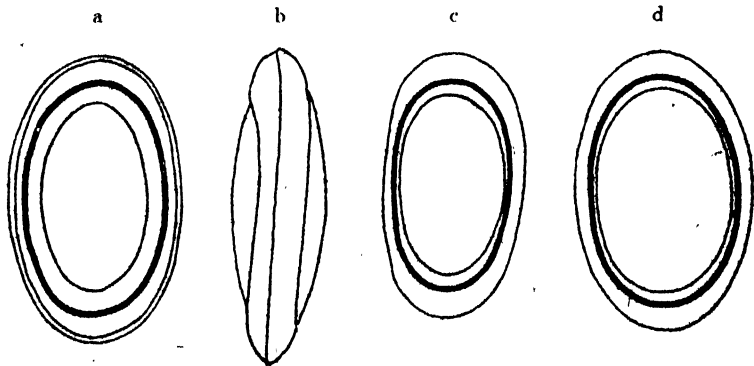
The zooecia are entirely recumbent, and thus the distal part of each zooecium is seldom bent upwards.

Two kinds of free statoblasts are observed. The one which is provisionally named type *casmiana* is very long in proportion to the breadth, being from 0.35 to 0.39 mm in length and from 0.17 to 0.21 mm in breadth (text-fig. 7, a, b).

It is of a pale yellow hue and



Text-fig. 5. Two statoblasts of *P. repens* var. *flabellum* of type *casmiana*. ($\times 100$)



Text-fig. 6. Statoblasts of *P. repens* var. *flabellum* of type *typica*, a—dorsal view; b—side view; c, d—ventral view. ($\times 100$)

looks almost colorless. The annulus is narrow and is of uniform breadth encroaching on the capsule on both faces.

All the air-cells are attached closely to the capsule. The capsule itself is transparent and is covered with scattered minute tubercles.

The other, which is provisionally named type *typica*, is of a yellowish brown color, being usually elongate and sometimes broadly elliptical.

The annulus encroaches on the capsule much more than in the case of type *casmiana*, being narrower on the sides than at the ends (text-fig. 8, a-d).

The length of the statoblasts of this type is between 0.34 and 0.38 mm, and the breadth is between 0.2 and 0.21 mm. The capsule is from 0.28 to 0.3 mm long and from 0.16 to 0.18 mm wide.

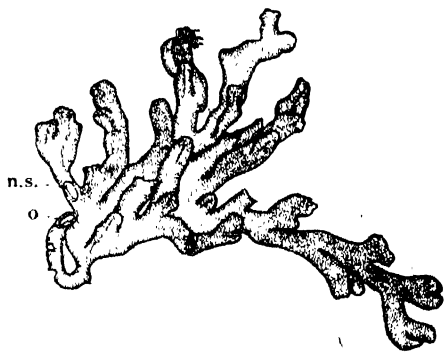
The free statoblasts of these two types are not always found in the same zoarium. Usually they are present in the different zoaria.

In one case (text-fig. 7) the two valves belonging to a statoblast of type *casmiana* were found adhering to the base of a zoarium, which had newly formed a number of statoblasts of type *typica*. Two zoaria possessing the statoblasts of different types are the same in external appearance.

In Tyôsen, the Tôhoku and Kantô districts of Japan, the zoaria which have the statoblasts of type *casmiana* were found rather rarely compared with the other. In the Tôhoku and Kantô districts, if the statoblasts of the one type are found in one pond or pool, there may also be seen statoblasts of the other type at the same time.

In form and size the fixed statoblasts of the two types above mentioned are similar to those of *P. repens* var. *emarginata*.

This variety was secured from Kôho and Ryûko, Kankyonandô.



Text-fig. 7. Small zoarium of *Plumatella repens* var. *flabellum*. o—the statoblast from which the zoarium first germinated. It is a statoblast of type *casmiana*. n.s.—newly formed statoblast of type *typica*.

6) *Hyalinella punctata* (HANCOCK)

Plumatella punctata, HANCOCK, 1850, p. 200, Pl. V, figs. 6-7, Pl. VI, fig. 1; ALLMAN, 1856, pp. 100-102, Fig. 15; HARTMEYER, 1909, p. 59, Fig. 116; ANNANDALE, 1910, p. 52; 1919, p. 94; ROUSSELET, 1916, p. 141.

Plumatella vesicularis, JULLIEN, 1885; VANGEL, 1894, p. 155.

Hyalinella punctata, HASTINGS, 1929, p. 303; ROGICK, 1935, p. 251; 1940, pp. 196-198, Pl. II, figs. 6-10, Pl. V, fig. 25; TORIUMI, 1941, p. 204, Fig. 8.

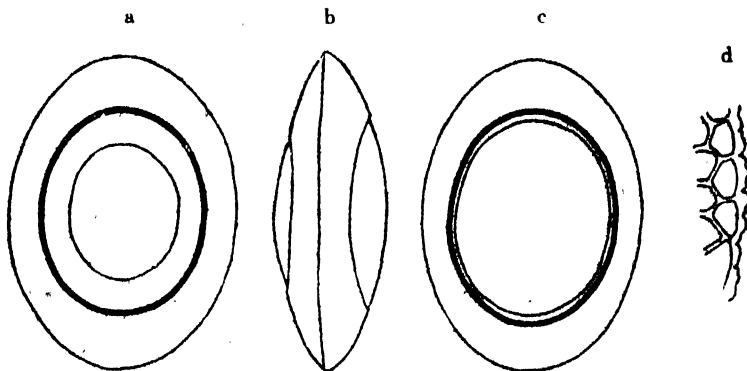
The zoarium forms a hyaline layer which is uniformly flat. The ectocyst is soft, more or less elastic, swollen and transparent.

The tips of the zooecia are rounded but are lacking in white spots.

The number of tentacles is between 41 and 46.

Free statoblasts only are produced, and they are broad and nearly circular.

The margin of the annulus is irregularly serrated and is observable when it is strongly magnified. The annulus covers both faces of the capsule but leaving a large area in the center. The capsule is minutely mammillated and is of brown color. The whole length of the free statoblasts is from 0.37 to 0.42 mm, and the breadth is from 0.29 to 0.31 mm. The capsule is from 0.26 to 0.33 mm long and from 0.22 to 0.24 mm wide.



Text-fig. 8. Statoblasts of *Hyalinella punctata*. a—dorsal view; b—side view; c—ventral view; d—serration on margin. (a-c, $\times 100$ d, $\times 450$)

This specimen dealt with in the present report seems to correspond to the form mentioned by Kraepelin as Var. *densa*. This form seems to be widely distributed in Tyôsen as the statoblasts were found at various places.

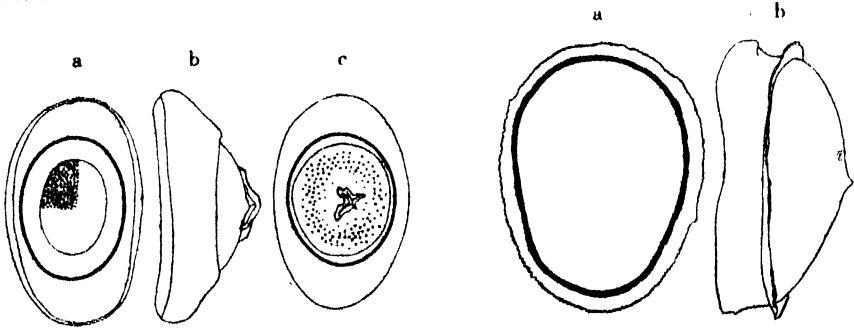
The specimens of this species were collected from Ryûko, Kankyô-nandô and Zyôsyaku-tyosuiti, Keisyônandô.

7) *Hyalinella toanensis* HÔZAWA & TORIUMI

Hyalinella toanensis, HÔZAWA & TORIUMI, 1940, Fig. 6, Pl. fig. 4, pp. 431-432; TORIUMI, 1941, p. 205, Fig. 9.

The zoarium forms a nodular mass around the stem of some water plants.

The surface is smooth. The jelly is copious, and attains a thickness of nearly 1 cm. It is colorless and hyaline, but is somewhat hard and elastic.



Text-fig. 9. Free statoblasts of *Hyalinella toanensis*. ($\times 50$)

Text-fig. 10. Fixed statoblasts of *H. toanensis*. a—dorsal view; b—side view. ($\times 50$)

The number of tentacles varies from 53 to 62.

The free statoblasts are elongated and are somewhat rhombic with both extremities rounded. The annulus encroaches a little more on one face than the other, and is narrower on the lateral sides than at the extremities.

The annulus is not curved as in the case of *H. indica* ANNANDALE.

The capsule has a large blunt process in the center of one face, and is mammillated on both faces. The blunt process of the free statoblast is provided with a peculiarly formed appendage which is spinous and transparent. They may be easily detached from the process of the statoblast.

The length of the free statoblasts varies from 0.52 to 0.56 mm and the breadth is between 0.34 and 0.36 mm. The capsule is from 0.36 to 0.37 mm long and is from 0.28 to 0.3 mm wide.

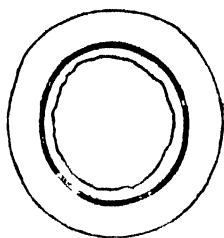
The fixed statoblasts are present. They are generally oval, but rarely circular in outline, and are almost black. They are provided with a serrated, stout chitinous lamella of a brown or black color. The capsule possesses a large process in the center of the dorsal surface, and it is

covered with scattered black tubercles. The fixed statoblasts are found adhering to the inner side of the zooecia. The capsule of the fixed statoblasts is from 0.56 to 0.6 mm in length and is from 0.44 to 0.52 mm in breadth. The chitinous lamella is 0.02–0.04 mm wide.

This species was found in Bukeiko, Kankyôhokudô.

8) *Stephanella hina* OKA

Stephanella hina, OKA, 1908, Pl. X, figs. 1–5, pp. 277–285; TORIUMI, 1941, p. 207, Fig. 10.



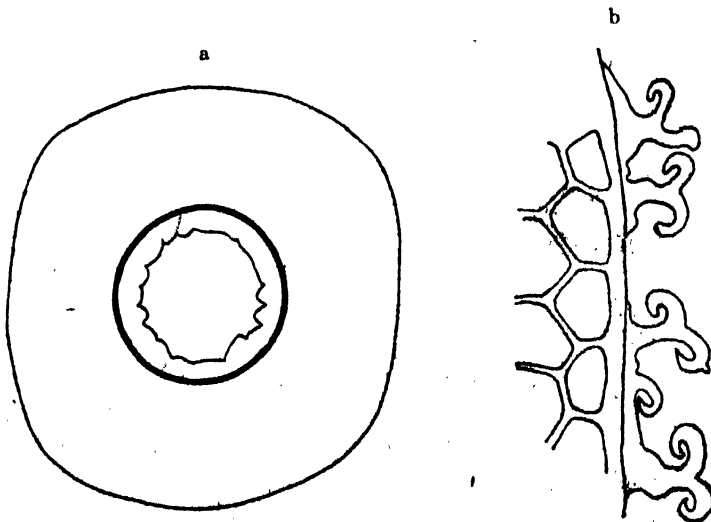
Text-fig. 11. Statoblast of *Stephanella hina*.

Only the free statoblasts were secured. They are circular and vary from 0.28 to 0.36 mm in diameter. The capsule ranges from 0.19 to 0.25 mm in diameter.

Localities are the following four small ponds: Atej, Zenrahokudô; Mikkaho, Kôgendô; Syuntôti and a small ponds in Keizyô-hu.

9) *Pectinatella gelatinosa* OKA

Pectinatella gelatinosa, OKA, 1890; 1907, p. 716, p. 117; ANNANDALE, 1907, p. 148; 1910, p. 56; ROUSSELET, 1919, p. 141; BORODIN, 1928, p. 488; HASTINGS, 1929, p. 305.



Text-fig. 12. Statoblast of *Pectinatella gelatinosa*. a—facial view; b—processes found on margin of annulus. (a, $\times 50$ b, $\times 600$)

Pectinatella burmanica, ANNANDALE, 1910, p. 56; ROUSSELET, 1919, p. 141; VORSTMAN, 1928, p. 12, Fig. 9, Pl. I, fig. 12; HASTINGS, 1929, p. 305.

Only the free statoblasts were obtained. They are nearly circular and are dark brown. The surfaces of the statoblasts are curved like a saddle. The annulus is large in proportion to the capsule and covers the latter slightly on both faces. Numerous processes are present surrounding the annulus, each bearing at its extremity a pair of hooks. The statoblasts of this species were found in Hokuti, in Reisentei, in Seiko, and in Sintaitei, Keikidô.

10) *Lophopodella carteri* (HYATT)

Lophopus sp., CARTER, 1859, p. 331.

Lophopodella carteri, ROUSSELET, 1907; VORSTMAN, 1928, pp. 10-12, Fig. 8, Pl. III, Fig. 11; LEE, 1936, pp. 399-401, Fig. 1; TORIUMI, 1941, p. 209, Figs. 12, 13.

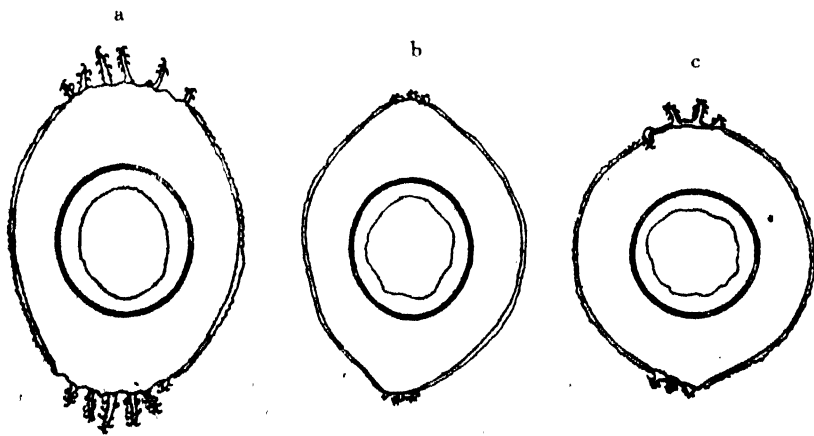
Lophopodella carteri var. *typica*, ROGICK, 1934, pp. 416-424; 1935, p. 250.

Pectinatella davenporti, OKA, 1907, pp. 117-120, Figs. 1, 2; 1907, p. 716; ANNANDALE, 1907, p. 148; SOILLAS, 1908, p. 268; ROUSSELET, 1916, pp. 141-142.

Lophopodella carteri davenporti, ANNANDALE, 1919, p. 97.

Lophopodella carteri var. *davenporti*, HASTINGS, p. 305; ROGICK, 1934, p. 420.

The zoarium is small and sacciform. The number of tentacles is between 73 and 77. Only the free statoblasts are seen. They are flat, sometimes curved in a saddle-like manner, sometimes spindle-shaped or sometimes more or less elliptical (rarely almost circular), and are sub-truncated at the ends.



Text-fig. 13. Statoblasts of *Lophopodella carteri*. ($\times 50$)

The length of the statoblast extruding the spines varies from 0.84 to 0.9 mm and the breadth is from 0.64 to 0.72 mm. The capsule is 0.42–0.46 mm long and 0.38–0.4 mm wide. Each of these spines bears on each side 3–11 (usually 5–6) small, semicircularly curved, flat, bluntly terminated barbs. On each side (excepting the extremities) of the annulus there may be seen an irregular minute serration.

The specimen of this species was secured from Rinti, Kankyômandô.

LITERATURE CITED

- ALLMAN, G. J., 1856. Monograph of Fresh-water Polyzoa.
- ANNANDALE, N., 1910. Materials for a revision of the Phylactolaematous Polyzoa of India. Rec. Ind. Mus. Vol. 5, pp. 37–57.
- , 1915. The Genus *Australella* and some allied species of Phylactolaematous Polyzoa. Ibid. Vol. 11, pp. 163–169.
- , 1919. Sponges, Hydrozoa and Polyzoa of Seistan. Ibid. Vol. 18, pp. 83–97.
- CARTER, H. J., 1859. On the Identity in Structure and Composition of the so-called seed-like Body of Spongilla with the Winter Egg of the Bryozoa. Ann. Mag. Nat. Hist. III: 3, pp. 331–343.
- DAHLGREN, U., 1934. A Species and Genus of Fresh-water Bryozoa new to North America. Science, Vol. 79, p. 510.
- DAVENPORT, C. B., 1904. Report on the Fresh-water Bryozoa of the United States. Proc. U. S. Nat. Mus. Vol. 27, pp. 211–221.
- HANCOCK, A., 1850. On the Anatomy of the Freshwater Bryozoa with Description of three Species. Ann. Mag. Nat. Hist. II: 5, pp. 173–204.
- HARMER, S. F., 1913. The Polyzoa of Waterworks. Proc. Zool. Soc. London. 1913. pp. 426–457.
- HARTMEYER, R., 1909. 'Bryozoa, Moostierchen' in Brauer's "Die Süßwasserfauna Deutschlands", 19, pp. 49–58.
- HASTINGS, B. A., 1929. Phylactolaematous Polyzoa from the "Pans" of the Transvaal. Ann. Mag. Nat. Hist. X: 3, pp. 129–137.
- HOZAWA, S. & TORIUMI, M., 1940. Freshwater Bryozoa of Manchoukuo. Rep. Limnobiol. Surv. Kwantung and Manchoukuo, conducted by Prof. T. KAWAMURA. pp. 425–432.
- KRAEPELIN, K., 1887. "Die Deutschen Süßwasser-Bryozoen".
- LEE, L., 1936. Notes on some Freshwater Polyzoa of Peiping. Sinensia, Vol. 7, pp. 399–407.
- OKA, A., 1891. Observations on Fresh-water Polyzoa (*Pectinatella gelatinosa*, nov. sp.). Journ. Coll. Sci. Tokyo Imp. Univ. Vol. IV, Part I, pp. 89–150.
- , 1906. A new species of Fresh-water Bryozoa. (*Pectinatella davenporti*), Zool. Mag. Tokyo, Vol. 18, pp. 307–310.
- , 1907. Eine dritte Art von Pectinatella (*Pectinatella davenporti* n. sp.). Zool. Anz. 31, pp. 716–718.
- , 1970. Zur Kenntnis der Süßwasser-Bryozoenfauna von Japan. Annot. Zool. Japan, Vol. VI, pp. 117–123.
- , 1908. Über eine neue Gattung von Süßwasserbryozoen (*STEPHANELLA* n. g.). Ibid. pp. 277–284.

- RAO, H. S., 1929. Sponges and Polyzoa of the Indawgyi Lake. Burma. Rec. Ind. Mus. Vol. 31, pp. 269-271.
- ROGICK, M. D., 1934. Studies on Freshwater Bryozoa. I. The Occurrence of *Lophopodella carteri* (HYATT) 1886 in North America. Trans. Amer. Micro. Soc. Vol. LIII, No. 4, pp. 416-424.
- , 1935. II. The Bryozoa of Lake Erie. Ibid. Vol. LIV, No. 3, pp. 245-263.
- , 1937. V. Some Addition to Canadian Fauna. Ohio Jour. Sci. Vol. XXXVII, No. 2, pp. 99-104.
- , 1940. IX. Additions to New York Bryozoa. Trans. Amer. Micr. Soc. Vol. LIX, No. 2, pp. 187-204.
- ROUSSELET, C. F., 1916. Statoblasts of Fresh-water Polyzoa. Journ. Roy. Micro. Soc. London, 1916, pp. 141-142.
- TAKAHASI, S., 1934. Sur *Lophopodella carteri* (HYATT) d'eau douce, originaire de Formosa. Annot. Zool. Japonese, Vol. 14, No. 3, pp. 347-350.
- TORIUMI, M., 1941. Studies on Freshwater Bryozoa of Japan. I. Sci. Rep. Tohoku Imp. Univ. Ser. 4, Vol. XVI, No. 2, pp. 193-215.
- VANGEL, E., 1894. Daten zur Bryozoen-Fauna Ungarns. Zool. Anz. 17, pp. 153-155.
- VORSTMAN, A. G., 1928. Some Fresh-water Bryozoa at West Java. Treubia, Vol. 10, pp. 1-14.

ON SOME LATER STAGES OF THE EMBRYOGENY OF COCCOPHORA LANGSDORFII¹⁾

By

MASATO TAHARA

Biological Institute, Tôhoku Imperial University, Sendai

(With 2 text-figures)

(Received June 26, 1911)

Growth by means of an apical cell is a most significant characteristic of the order Fucales. In the year 1931 NIENBURG²⁾ published a paper dealing with the origin of the apical cell of *Fucus vesiculosus*. According to his investigation the apical cell of this alga comes into existence by the transformation of the basal cell of one of the hairs growing at the apex of the embryo.

The present investigation was primarily undertaken to ascertain whether the same relation exists or not in higher members of the Fucales. The embryo of *Coccophora*, a genus closely related to *Sargassum*, is very suitable for such an investigation. At present only one species is known in this genus, namely *C. Langsdorfii*. This plant grows exclusively in the Japan Sea. The liberation of the germ cells occurs almost simultaneously in days of spring tide in early April. The material of the present investigation was obtained this year in the Asamushi Marine Biological Station and was cultured in the laboratory in Sendai.

In the early stages of its development the embryo consists of cells of equal size. But later a group of cells situated at the apex of the embryo becomes gradually larger than the neighbouring cells (Fig. 1, a). Each of these apex cells contains a large nucleus. Meanwhile they divide transversely and become two-celled. The basal cell of the two divides again also transversely. The same thing is repeated several times, the basal cell remaining always the dividing cell (Fig. 1, b). The group of hairs thus formed are concealed at first in a hollow, that is cryptostoma.

¹⁾ The cost of this research has been defrayed from the Scientific Research Expenditure of the Department of Education.

²⁾ W. NIENBURG, 1931: Die Entwicklung der Keimlinge von *Fucus vesiculosus* und ihre Bedeutung für die Phylogenie der Phaeophyceen. Wiss. Meeresuntersuchungen. Abt. Kiel. Bd. XXI.

But soon by their elongation they appear on the outside. In this stage of development the embryo of this plant has a close resemblance to that of *Fucus*. But in higher members of the order Fucales, as is already known, the first apical portion of the embryo is transformed later into a

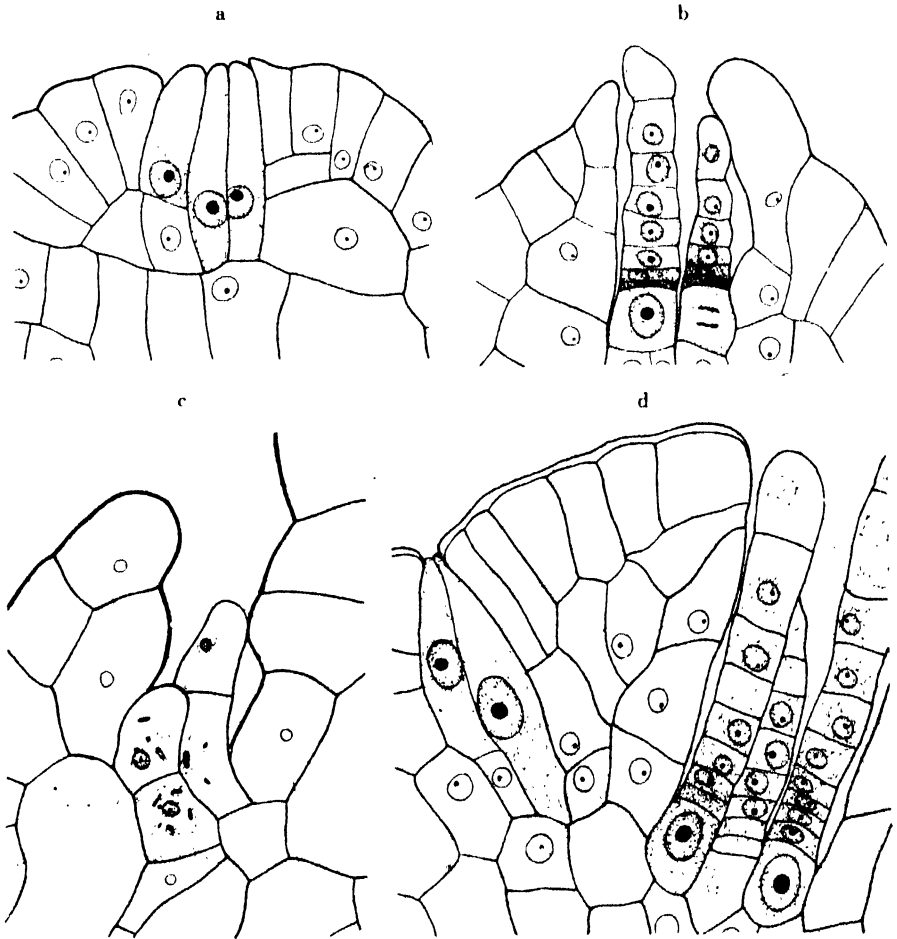


Fig. 1. a, apex of the embryo in an early stage of development. b, hairs are growing in the cryptostoma at the apex of the embryo. c, cryptostoma for the stem apex in its early development. d, cryptostoma in the first leaf of the embryo in two different stages of development. $\times 900$

leaf. And the apex of the stem is developed *de novo* on a lateral side of the embryo. So in these genera the apical cell of the stem cannot have any relation to the first cryptostoma produced in the embryo.

In *Coccophora Langsdorfii* the new growing point for the stem is completely developed in early July, at a point on a lateral side, about the middle of the entire length of the embryo. Before the formation of this growing point, however, a cryptostoma containing slender hairs is formed at the spot, where the growing point of the stem will be formed later. In the materials fixed in later May or early June we can follow the development of this cryptostoma. The cryptostomata which appear one after another in the leaf are all exogenous in their development (Fig. 1, d). But the cryptostoma for the stem apex is endogenous in its origin, the meristematic cells of this organ not being the cells lying in the outermost layer of the embryo. So in appearance this cryptostoma in its early development has a resemblance to the lenticel of the flowering plants (Fig. 1, c).

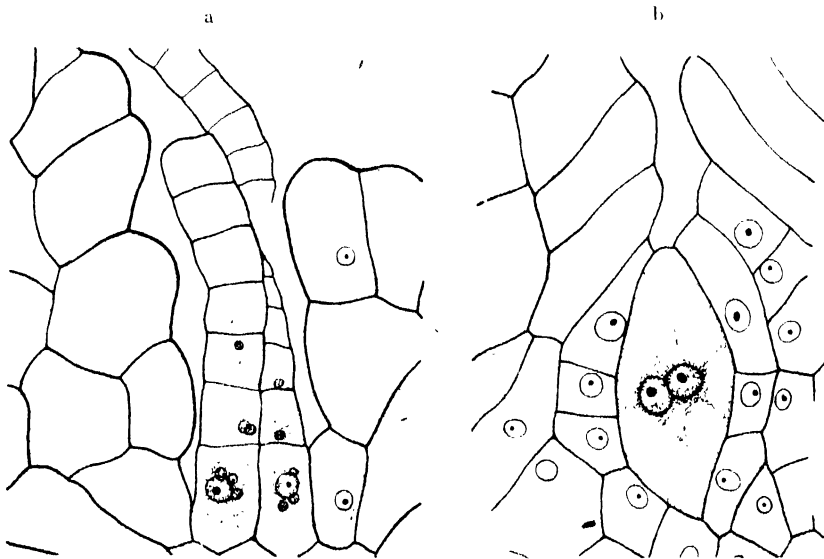


Fig. 2. a, cryptostoma for the stem apex. The cells of the distal portions of the hairs are already degenerating. b, single apical cell in the cryptostoma for the stem apex. $\times 900$

Moreover in the cryptostomata of the leaf, the basal dividing cells of the hairs have each a specially large nucleus. But the corresponding cells in the cryptostoma for the stem apex have a nucleus which does not differ in size from those of the usual somatic cells. Accordingly the hair cells produced by the divisions of those cells have also a very small

nucleus and soon degenerate, becoming empty (Fig. 2, a). But later one of the basal dividing cells in this cryptostoma grows larger and appears to become the apical cell of the stem (Fig. 2, b).

Thus the description given by NIENBURG as to the embryogeny of *Fucus vesiculosus* applies also in the essential points to *Coccophora Langsdorffii*.

In conclusion the writer wishes to express his thanks to Mr. K. ABE for his assistance during the course of this investigation.

SUMMARY

In a later stage of the embryonal development of *Coccophora Langsdorffii* a cryptostoma is produced endogenously at a point on a lateral side of the embryo, about midway of the entire length of the embryo. The hairs in this cryptostoma degenerate soon and one of the basal cells of the hairs grows larger and later becomes the apical cell of the main axis of the plant.

THE SEXUAL CHARACTER OF THE SEA URCHIN,
STRONGYLOCENTROTUS PULCHERRIMUS
A. AGASSIZ¹⁾

By

ISAO MOTOMURA

Biological Institute, Tôhoku Imperial University, Sendai

(Received July 18, 1941)

Only a few cases have been reported on the secondary sexual character of Echinoderms, as far as the writer is aware. In *Asterina gibbosa*, *Asteracanthion* and *Ophiothrix petersi* the sexual dimorphism in coloration has been observed²⁾. In *Cucumaria frondosa* the form of the genital papillae is different in both sexes³⁾.

While working on the embryology of the sea urchin, *Strongylocentrotus pulcherrimus*, the writer found a sexual dimorphism of this species. The individuals having white tube feet on the oral side are females, and those having yellow tube feet are females. Although the coloration of the tube feet shows marked individual variations, it is easily distinguished in the living individuals attaching on the wall of a glass vessel filled with sea water. And the individuals showing an intermediate color tone are relative few in number. The color of the shell and spines, on the other hand, has no remarkable correlation to the sexes, showing many individual variations between dark green and light greyish purple.

As already stated by the writer, the egg of this species contains orange yellow pigment, which is easily extracted with acetone⁴⁾. The pigment of the tube feet seems to be identical with that of the egg, because it is also easily soluble in acetone. The amounts of the pigment contained in the dried shells of both sexes were compared quantitatively with acetone extracts, and as it was expected, the shell of the male contained a less amount of the yellow pigment in comparison with that of the female. The writer does not know the physiological meaning of the pigment in this species. But the sexual distinction in the external character of this species will be useful for the embryological practice.

¹⁾ Aided by the Scientific Research Expenditure of the Department of Education.

²⁾ BREHMS Tierleben. 4 Aufl. 4. Leipzig (1912).

³⁾ EDWARDS, C. L. Holothurioidea. 1 *Cucumaria frondosa*. Zool. Jahrb. Syst. 29. (1910).

⁴⁾ MOTOMURA, I. Determination of the embryonic axis in the eggs of Amphibia and Echinoderms. Sci. Rep. Tôhoku Imp. Univ. Biol. 10. (1935).

**ARTIFICIAL INSEMINATION OF THE EGGS OF THE
STAR FISH, *ASTERINA PECTINIFERA*
(MULLER ET TROSCHEL)¹⁾**

By

ISAO MOTOMURA

Biological Institute, Tôhoku Imperial University, Sendai

(Received July 18, 1941)

The egg of the star fish is spawned usually before the beginning of the maturation division, which proceeds in sea water. The sperm enters into the egg during the stage of metaphase of the first maturation division. The artificial insemination is, therefore, usually carried out after the dissolution of the germinal vesicle. In *Asterina pectinifera* the egg cannot be fertilized at a good rate, when the egg is taken out by cutting the ovary in sea water. Last autumn the writer found that one of the factors inhibiting the dissolution of the germinal vesicle is the presence of the follicle epithelium on the egg surface.

The ovarian egg is covered with a thin membrane, the follicle epithelium, of 5μ in thickness. In most of the eggs, which are taken out by cutting the ovary in sea water, the follicle epithelium remains intact. And in a few eggs, which have lost the membrane, the germinal vesicle soon disappeared. On the suggestion of this observation, the writer tried to remove the membrane. And by shaking the egg in a test tube with sea water, the membrane was removed from most eggs without injury. The germinal vesicle soon disappeared. And 30 minutes after the shaking more than 90 per cent of the eggs could be fertilized, forming a beautiful fertilization membrane, while the eggs, which were not shaken could not be fertilized, having a distinct germinal vesicle. In this case, therefore, the presence of the follicle epithelium is not simply an obstacle to the entrance of the sperm, but it is the inhibitor of the maturation division, which is stimulated by coming in direct contact with sea water. It is suggested from this fact that in the natural process of spawning of this species the follicle epithelium may be removed mechanically at the time of passing through a small opening of the genital pore.

¹⁾The cost of this research has been defrayed from the Scientific Research Expenditure of the Department of Education.

ON THE STRUCTURE OF THE CONCEPTACLE OF *SARGASSUM* AND *COCCOPHORA**

By

MASATO TAHARA

Biological Institute, Tôhoku Imperial University, Sendai

(With Plate XXVI and 2 text-figures)

(Received July 26, 1941)

Both in Europe and the United States of America *Fucus* is the most common representative of the order Fucales. In Japan *Fucus* grows only in Hokkaido, but more advanced members of the order, *Sargassum*, *Coccophora* and *Cystophyllum* are commonly found along the coasts of the four main islands. At present, however, our knowledge as to these genera still leaves much to be desired. In previous papers the present writer described the conceptacle development of these plants. In the present paper the structures of the fully developed conceptacle of these algae will be dealt with. Generally the materials were fixed with osmium-chrom-acetic solution. Sections, mostly 20 in thickness, were stained with HEIDENHAIN's iron alum haematoxylin and light green.

1. *Sargassum Horneri* AG.

In a paper published in 1913 the present author wrote on the structure of the conceptacle of this plant as follows: "Generally speaking, the paraphyses of *Sargassum* do not protrude from the conceptacle as they do in *Fucus*. In *S. Horneri* they compose a disklike plug at the opening of the conceptacle. In a few seconds after immersion in the mixture medium above mentioned, the plug comes out slowly, with some broken pieces of paraphyses (text-fig. 1); and then the conceptacle commences to discharge its oogonia one after another."

To elucidate the structure above mentioned a microphotograph is given in Pl. XXVI, fig. 1. The disk-shaped plug is composed essentially of a gelatinous substance, in which the distal ends of the paraphyses are embedded. The plug of this kind is found, however, only at the ostiole

*The cost of this research has been defrayed from the Scientific Research Expenditure of the Department of Education.

of the conceptacle of the female individual. In the male conceptacle the same structure does not exist at all (text-fig. 2).

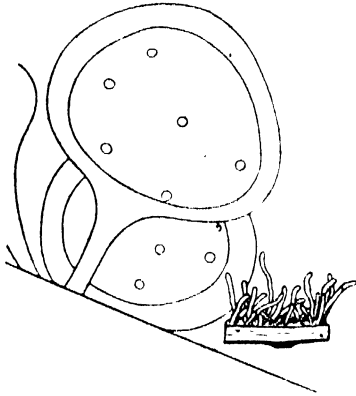


Fig. 1. *Sargassum Horneri*. Oogonia and plug of the conceptacle attached on the outer surface of the receptacle. $\times 140$ (After TAHARA, 1913.)



Fig. 2. *Sargassum Horneri*. Median section through a male conceptacle. $\times 110$

2. *Sargassum tortile* Ag.

The material for investigation of this species was collected on June 10th of this year in Asamushi. A part of a cross section of the female receptacle of this plant is shown in Pl. XXVI, fig. 2. An irregular mass of gelatinous substance is seen at the ostiole of the conceptacle. But in this case the connection between the paraphyses and the gelatinous substance is not so close as in *Sargassum Horneri*. In preparations, the paraphyses are seen generally detached from the gelatinous substance; this is caused by the shrinkage of the gelatinous substance. Before the liberation of the oogonia the gelatinous mass must be naturally thrown off to the outside. But on that occasion no damage is brought about in the terminal portions of the paraphyses.

3. *Sargassum fusiforme* (HARV.) SETCH. (=*Hizikia fusiformis* OKAMURA)

The material for investigation of this plant was collected on July 9th of this year in Misaki. The structure of the female conceptacle is just the same as that of *S. tortile*. A stopper of an irregular shape is found

at the ostiole of the conceptacle (Pl. XXVI, fig. 3). The same structure is seen also in the female conceptacles of *S. Ringgoldianum* HARV., *S. Thunbergii* O. KUNTZE, *S. enerve* AG. and *S. hemiphyllum* AG.* Thus the structure found in *S. Horneri* appears to be peculiar to this species.

1. *Sargassum patens* AG.

The seven species of *Sargassum* above described all belong to the subgenus *Bactrophycus*. *Sargassum patens* AG., however, is a species belonging to the subgenus *Schizophycus*, a more primitive subgenus. The conceptacle development of this subgenus is totally different from that of the subgenus *Bactrophycus* (TAHARA, 1941, a). So it is desirable to know the structure of the conceptacle of this species. Specimens of this plant were collected also on July 9th of this year in Misaki. A section through the female conceptacle is shown in Pl. XXVI, fig. 4. No stopper is found at the ostiole. The formation of a stopper at the ostiole of the conceptacle seems to be a characteristic existing only in the species of higher organization among the genus *Sargassum*.

5. *Sargassum micracanthum* YENDO

Sargassum micracanthum is a species of the subgenus *Micracantha*. The conceptacle development of this alga proceeds in a manner just intermediate between the primitive and the *Bactrophycus*-type (TAHARA, 1941, b). So it will be interesting to know the structure of the fully developed conceptacle of this plant. The material was obtained on June 5th of this year in Kuwagawa, Niigata Prefecture. To the writer's astonishment, cross sections through the female receptacle of this plant have shown two kinds of conceptacle. In the one the structure is just the same as seen in most species of *Bactrophycus*, while in the other the paraphyses have grown extensively through the ostiole of the conceptacle (Pl. XXVI, fig. 5). In the writer's opinion, the former may perhaps be a state still not attaining to full maturity. As is already known (TAHARA, 1913), in *Cystophyllum sisymbrioides* the paraphyses in the female conceptacle suddenly begin to grow at the time of oogonia liberation. In this point *S. micracanthum* appears to have a resemblance to this species.

*The materials for investigation of *S. Thunbergii* and *S. hemiphyllum* were sent by Mr. K. ABE in Asamushi. The writer wishes to express best thanks to him.

6. *Coccophora Langsdorfii* GREV.

In the genus *Coccophora* only one species is known at present, namely *C. Langsdorfii*. The material for investigation of this plant was collected on April 10th of this year in Asamushi. A cross section of a receptacle of a female plant is shown in Pl. XXVI, fig. 6. In this plant, too, no gelatinous mass at all is seen at the ostiole.

SUMMARY AND CONCLUSION

1. In the previous papers concerning the conceptacle development of a number of species of *Sargassum* the present writer has concluded that in the species of *Sargassum* of lower organization the tongue cell of the conceptacle remains attached to the wall of the conceptacle through the whole course of development, while in those of higher organization the tongue cell becomes free from the wall of the conceptacle and is transferred to the ostiole to close up the passage to the outside. Although the tongue cell cannot be detected, the same relation can be found also in the fully developed conceptacle. Namely, in the species of higher organization a stopper of gelatinous substance is formed at the ostiole of the female conceptacle; such a structure cannot be found in the conceptacle of the species of lower organization.

2. *Sargassum Horneri*, a species of the subgenus *Bactrophycus*, in several points distinctly differs from other members of the same subgenus. The stopper found in the female conceptacle of this species is disk-shaped. The distal portions of the paraphyses are firmly embedded in the substance of the stopper. And before the liberation of oogonia the stopper is ejected to the outside, with some broken pieces of the paraphyses. In the other species hitherto studied the stopper is only a mass of irregular shape; the connection between the mass and the paraphyses is not so strong as that in *Sargassum Horneri*. Thus the terminal portions of the paraphyses becomes easily detached from the stopper.

3. In *Sargassum micracanthum* YENDO, a species of the subgenus *Micracantha*, the paraphyses in the female conceptacle vigorously grow to the outside through the ostiole before the occurrence of the oogonia liberation.

LITERATURE

1. TAHARA, M. 1913. Oogonium Liberation and the Embryogeny of Some Fucaceous Algae. Journ. Coll. Sci. Imp. Univ. Tokyo. Vol. XXXII.
2. TAHARA, M. 1940. On the Development of the Conceptacle of *Sargassum*, *Coccophora* and *Cystophyllum*. Sci. Rep. Tōhoku Imp. Univ. Vol. XV. No. 3.
3. TAHARA, M. 1941. a. Further Studies of the Conceptacle Development of *Sargassum*. Sci. Rep. Tōhoku Imp. Univ. Vol. XVI. No. 1.
4. TAHARA, M. 1941. b. Conceptacle Development of two Species of *Sargassum* of the Subgenus *Micracantha*. Sci. Rep. Tōhoku Imp. Univ. Vol. XVI. No. 4.

EXPLANATION OF PL. XXVI.

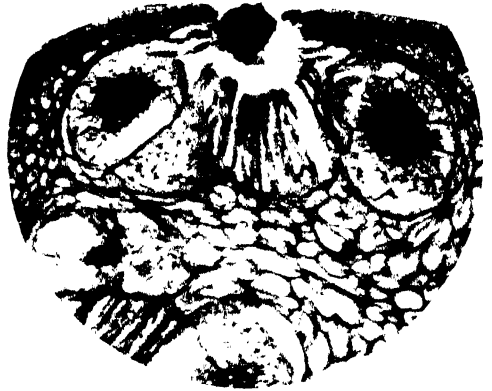
Median sections through the female conceptacle. Magnification. $\times 95$.

1. *Sargassum Horneri* AG.
2. *Sargassum tortile* AG.
3. *Sargassum fusiforme* (HARV.) SETCH.
4. *Sargassum patens* AG.
5. *Sargassum micracanthum* YENDO
6. *Coccophora Langsdorfi* GREV.

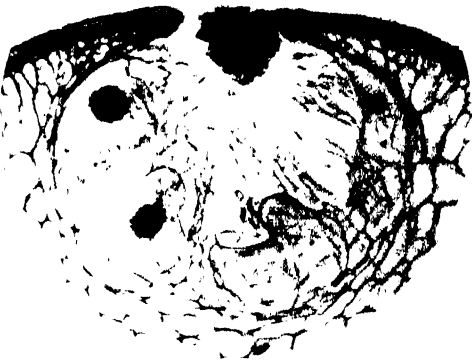
1



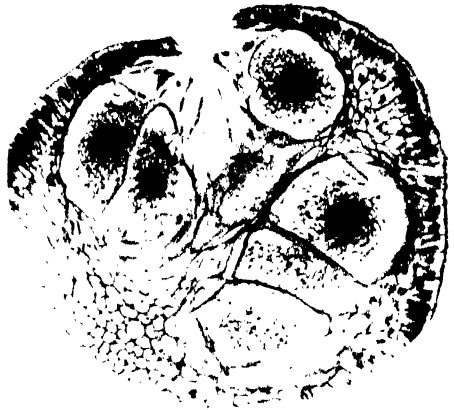
2



3



4



5



6



WEITERE UNTERSUCHUNGEN ÜBER DIE BEFRUCHTUNG VON COCCOPHORA UND SARGASSUM^{1), 2)}

VON

KÓGORÓ ABE

Biologisches Institut der Kaiserlichen Tōhoku Universität, Sendai, Japan

(Mit Taf. XXVII-XXX)

(Eingegangen am 9. August 1941)

Da meine früheren Angaben über die Befruchtung von *Coccophora Langsdorfii* (1932) und *Sargassum tortile* (1938) ausschliesslich auf den mit den fixierten Materialien ausgeführten Untersuchungen beruhten, habe ich sie wieder dieses Jahr in lebenden Materialien studiert. Und darauf erfolgte die Entdeckung einiger etwas merkwürdigen Tatsachen, worüber ich unten kurz mitteilen möchte.

1. *Coccophora Langsdorfii* (TURN.) GREV.

Gleich nach der künstlichen Besamung bemerkt man unter dem Mikroskop viele an der Oogoniumwand klebende Spermatozoiden. Inzwischen treten einige von ihnen in das Innere des Oogoniums ein und schwimmen etwa 10 bis 20 Minuten aktiv um die Eizelle. Wenn ein Spermatozoid in der vom Augenfleck umgekehrten Seite mit der Eizelle verschmilzt, so tritt eine papillenartige Vorwölbung (Taf. XXVII. Fig. 2-5), ein sogenannter Empfängnishügel, an der Stelle auf, wo das Spermatozoid in die Eizelle eindringt. Ein einziges Beispiel im Pflanzenreich wurde von KNAPP im Jahre 1931 bei *Cystoseira barbata* gefunden. Nach seiner Beschreibung scheint aber der Versuch bei dieser Pflanze etwas schwer zu sein; es ist ihm nur einmal gelungen einen solchen Hügel zu finden. Glücklicherweise liegt keine besondere Schwierigkeit in der Beobachtung dieser Erscheinung bei *Coccophora Langsdorfii* vor. Vielmals habe ich sie bei dieser Pflanze ganz klar verfolgen können. Nach der Beschreibung von KNAPP, verändert sich bei seiner Pflanze die ganze Oberfläche der Eizelle kurz nach dem Eindringen des Spermatozoids unregelmässig

¹⁾ Contribution from the Marine Biological Station, Asamushi, Aomori, No. 175.

²⁾ Diese Forschung wurde auf Kosten der Ausgaben des Unterrichtsministeriums für wissenschaftliche Forschung ausgeführt.

rauh und nachher wird sie allmählich wieder glatt. Eine solche Erscheinung konnte ich aber bei meinem Objekt nicht ersehen.

KNAPP hat in seiner Untersuchung von *Cystoseira* noch eine bemerkenswerte Erscheinung gefunden. Nämlich, beim Keimungsversuche im Dunkeln entsteht das Rhizoid des Embryos immer an der Stelle, wo vorher der Empfängnishügel vorhanden war. Um diese Erscheinung bei meinem Material zu bestätigen, verfuhr ich folgendermassen. Die Befruchtung wurde unter dem Mikroskop auf einem Objektträger verfolgt und die Stelle, wo der Empfängnishügel auftrat, wurde auf einer Skizze durch einen Pfeil markiert. Etwa 20 Minuten nach Befruchtung wurde der Objektträger in eine Schale mit Seewasser gelegt; es wurde aber dabei nicht verdunkelt.

Nach dem völligen Eindringen des Spermatozoids in die Eizelle nimmt die Höhe des Empfängnishügels meistens etwas ab. Aber dann spitzt sich das Ei allmählich in der Richtung des Hügels zu (Taf. XXVII, Fig. 6). Der Zustand des Eies in etwa 5 Stunden nach der Besamung ist in Taf. XXVIII, Fig. 7 gezeigt worden. Nach etwa 20 bis 24 Stunden wird das Ei zuerst durch eine Querwand in zwei Zellen geteilt (Taf. XXVIII, Fig. 9). Einige darauffolgende Stadien sind in Figg. 8–10 vergegenwärtigt worden. Am Ende möchte ich noch hinzufügen, dass ich öfters zwei Vorwölbungen in ein und derselben Eizelle ersehen konnte. Diese beiden dürften allem Anschein nach Empfängnishügel sein. Von solchen Eiern entstehen wahrscheinlich die doppelpoligen Embryonen, die nicht selten in der Tat beobachtet werden.

2. *Sargassum tortile* C. Ag.

In meiner früheren Untersuchung über die Befruchtung dieser Art gebrauchte ich die nur im natürlichen Standort befruchteten Eier. Aber es stellte sich heraus, dass zum genaueren Studium der Befruchtungsvorgänge künstliche Besamung auch bei dieser Alga ganz notwendig ist. Nach KUNIEDA (1941), treten die Eier von *Sargassum Horneri* beim Versuch im Laboratorium nur im in fließendem Seewasser stehenden Material aus den Konzeptakeln aus; beim in stagnierendem Seewasser enthaltenen Material findet das Austreten der Eier nie statt. Aber bei *Sargassum tortile* können wir eine normale Entleerung der Eier auch im in einer Schale stillstehenden Material ganz leicht beobachten, wenn das Material schon vollgereift ist. Dies ist auch der Fall bei *Coccolophora Langsdorffii*.

Nach der künstlichen Besamung konnte ich auch bei *Sargassum tortile*, wie bei *Coccophora*, eine papillenartige Vorwölbung auf der glatten Oberfläche der Eizelle bemerken. Wie schon wohl bekannt, erhalten die Eier von *Sargassum* zuerst acht Kerne. In der Nähe des Hügels kann man meistens deutlich einen Kern ersehen, mit welchem der Spermakern sich vereinigen soll. Die Verfolgung des weiteren Verlaufs der Entwicklung leitet uns merkwürdigerweise auch bei dieser Art zum Schluss, dass der Rhizoidpol des Embryos an der Stelle, wo der Empfängnishügel vorhanden war, gebildet wird. Wie bei *Coccophora*, kann man gelegentlich zwei Empfängnishügel in ein und derselben Eizelle ersehen. Also liegt die Vermutung nahe, dass von solchen Eiern die sogenannten Doppel-embryonen, wie sie TAHARA (1927) seinerzeit abgebildet hat, entstehen.

ZUSAMMENFASSUNG

1. Die Entleerung der Eier findet bei *Sargassum tortile* wie bei *Coccophora Langsdorfi* auch im Laboratorium im stillstehenden Seewasser statt.
2. Bei diesen beiden Arten konnte ich den deutlichen Empfängnishügel dort bemerken, wo das Spermatozoid in die Eizelle eingedrungen ist.
3. Der Rhizoidpol wird an der Stelle gebildet, wo der Empfängnishügel vorher vorhanden war.

Es ist mir vor allem ein Bedürfnis, an dieser Stelle meinem hochverehrten Lehrer, Herrn Prof. Dr. M. TAHARA, auf dessen Anregung und unter dessen Leitung die vorliegende Arbeit ausgeführt wurde, meinen herzlichsten Dank für seine vielen Ratschläge während der Untersuchung auszusprechen.

LITERATURVERZEICHNIS

- 1) ABE, K. 1938. Über die Befruchtung und die ihr folgende erste Kernteilung bei *Sargassum*. Sci. Rep. Tōhoku Imp. Univ., Biol. Vol. 13. No. 3.
- 2) KNAPP, E. 1931. Entwicklungsphysiologische Untersuchungen an Fucaceen-Eiern. I. Zur Kenntnis der Polarität der Eier von *Cystoseira barbata*. Planta Bd. 14.
- 3) KUNIEDA, H. and SUTO, S. 1940. On the Fertilization in *Sargassum Horneri* Ag. Japanese Journal of Botany, Vol. 11.
- 4) TAHARA, M. 1927. Experiments on the Eggs of *Sargassum*. Bot. Mag. Vol. XLI, No. 483.
- 5) TOMITA (ABE), K. 1932. Befruchtung und Kernteilung bei *Coccophora Langsdorfi* (TURN.) GREV. Sci. Rep. Tōhoku Imp. Univ., Biol., Vol. 7.

TAFELERKLÄRUNG

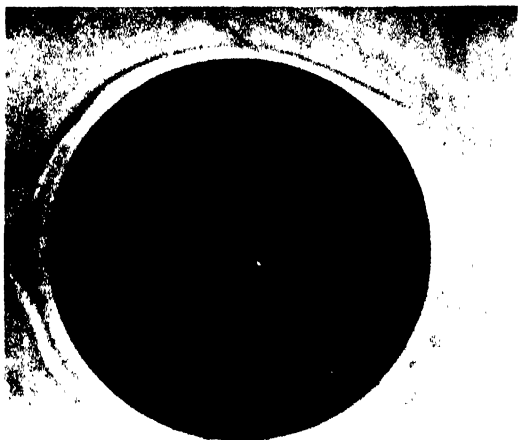
Alle Mikrophographien wurden mit Hilfe von LEITZ Makam aufgenommen:
Taf. XXVII, Fig. 1-6, $\times 230$, Taf. XXVIII, Fig. 7-12, $\times 180$, Taf. XXIX, Fig. 1-3, $\times 140$,
Fig. 4, $\times 240$, Taf. XXX, Fig. 5-8, $\times 140$.

TAFELN XXVII-XXVIII.

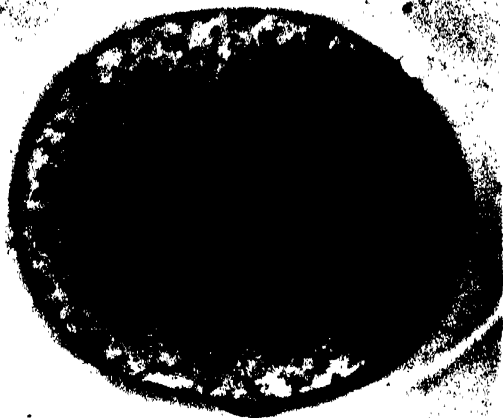
Fig. 1. Unbefruchtetes Ei. Fig. 2-5. Papillenartige Vorwölbungen an den befruchteten Eiern. Fig. 6. Etwas gespitztes Ei. Fig. 7. Sehr gespitztes Ei. Fig. 8. Mit Zellhaut umgehülltes Ei. Fig. 9. Zweizelliger Embryo. Fig. 10. Dreizelliger Embryo. Fig. 11. Vierzelliger Embryo. Fig. 12. Zwei Empfängnishügel in ein und derselben Eizelle.

TAFELN XXIX-XXX.

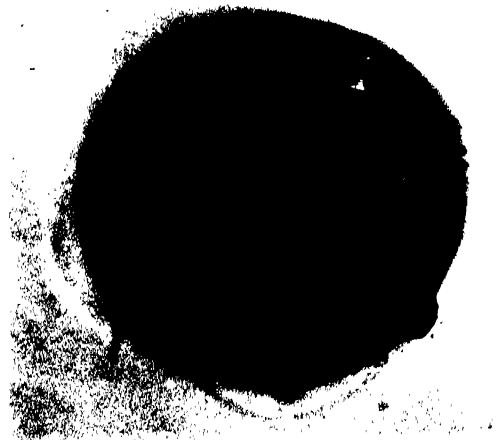
Fig. 1. Unbefruchtetes Ei. Fig. 2-4. Empfängnishügel der befruchteten Eier. Fig. 5. Das befruchtete Ei ist etwas gespitzt worden. Fig. 6. Zweizelliger Embryo. Fig. 7. Dreizelliger Embryo. Fig. 8. Vierzelliger Embryo.



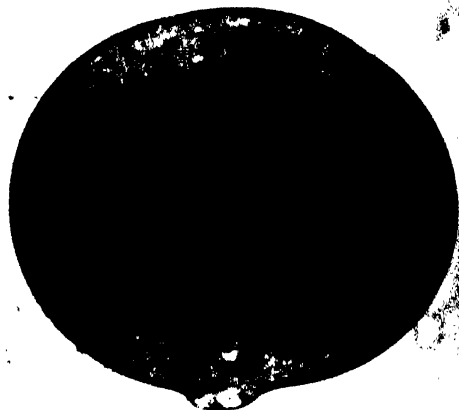
1



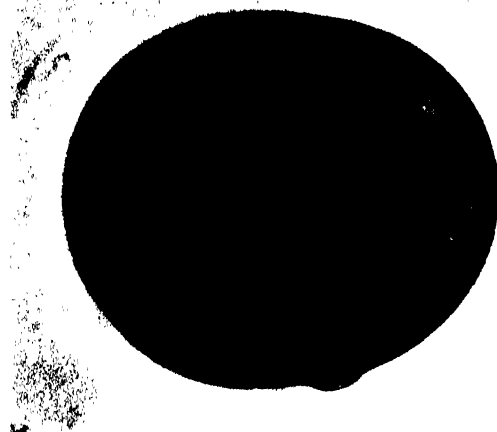
2



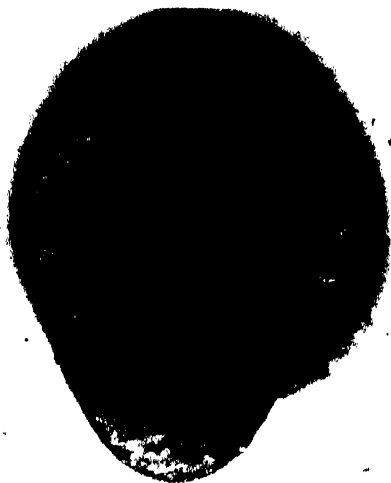
3



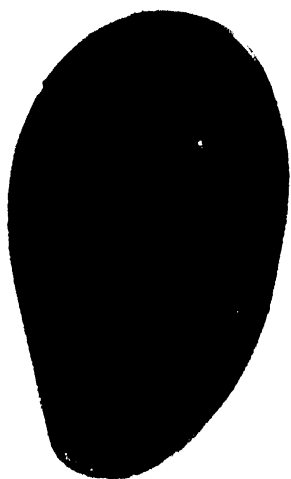
4



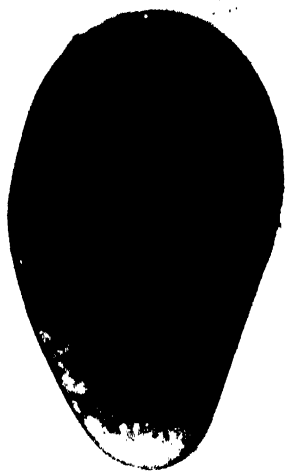
5



6



7



8



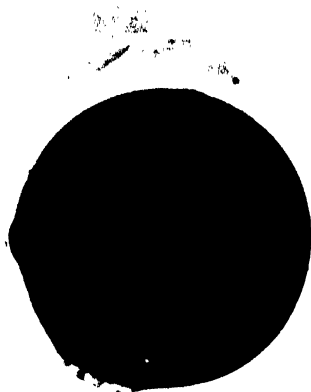
9



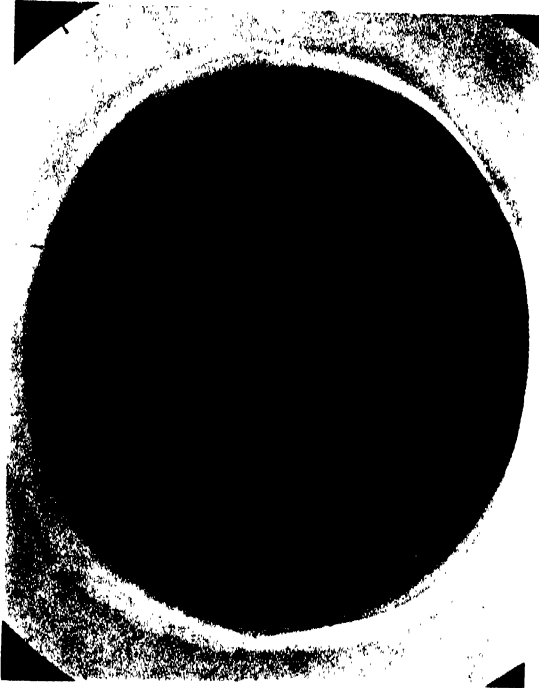
10



11



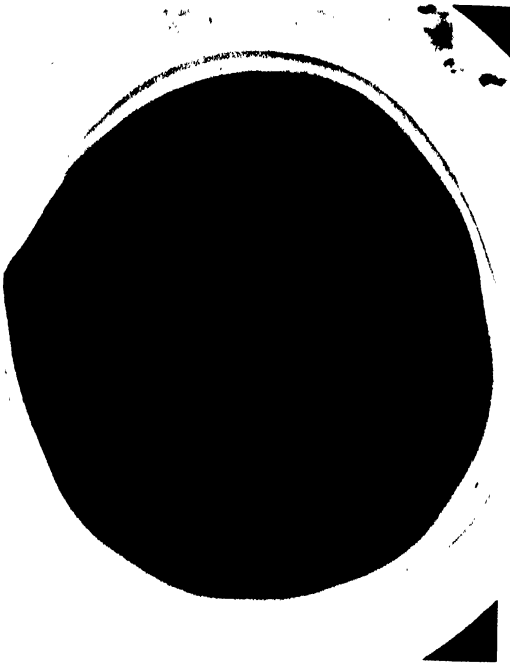
12



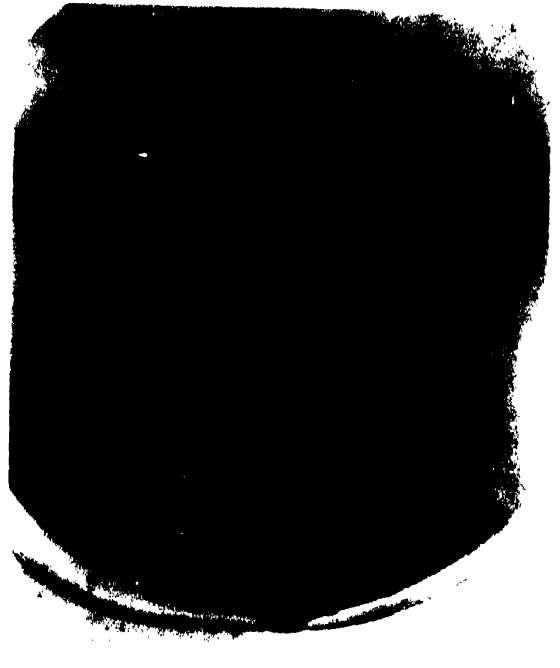
1



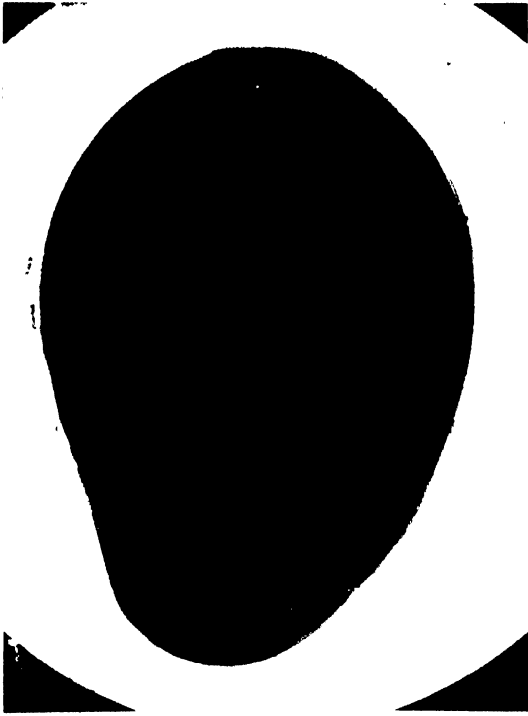
2



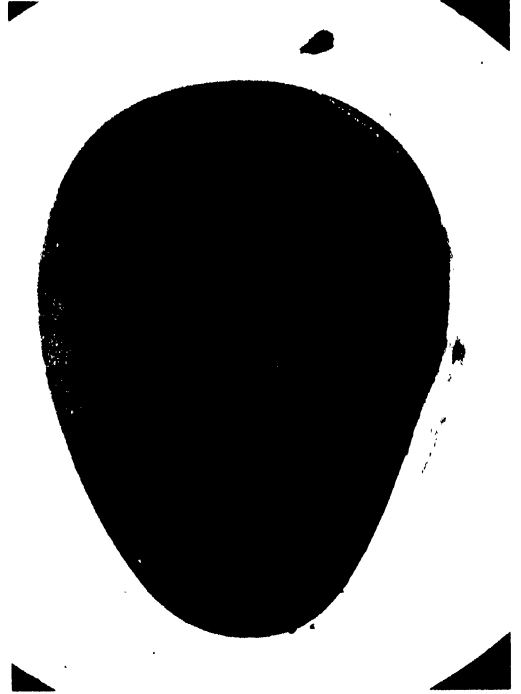
3



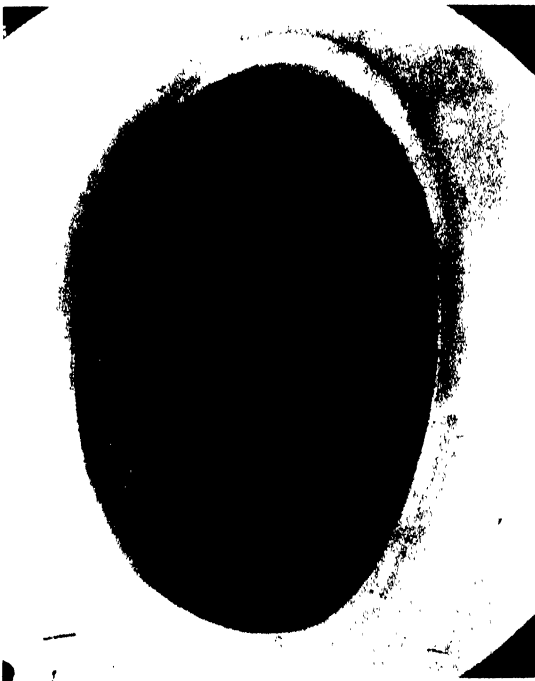
4



5



6



7



8

I. A. B. I. 75.

IMPERIAL AGRICULTURAL RESEARCH
INSTITUTE LIBRARY
NEW DELHI.

[illegible]